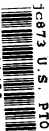


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UTILITY PATENT APPLICATION TRANSMITTAL UNDER 37 CFR §1.53(b)

Attorney Docket Number 07678/062004

Applicant Hai-Ying Zhu et al.

Title GRAPEVINE LEARFROLL VIRUS (TYPE 2) PROTEINS AND THEIR USES

PRIORITY INFORMATION:

This application is a continuation of and claims priority from United States patent application 09/080,983, filed May 19, 1998, (now pending), which claims priority from U.S. patent application 60/047,194, filed May 20, 1997(abandoned). This work was supported by the U.S. Department of Agriculture Cooperative Grant No. 58-2349-9-01. The U.S. Government may have certain rights in the invention.

APPLICATION ELEMENTS:

Cover sheet	1 page
Specification	79 pages
Claims	2 pages
Abstract	1 pages
Drawing	14 sheets
Combined Declaration and POA, which is: <input type="checkbox"/> Unsigned; <input type="checkbox"/> Newly signed for this application; <input checked="" type="checkbox"/> A copy from prior application 09/080,983 and the entire disclosure of the prior application is considered as being part of the disclosure of this new application and is hereby incorporated by reference therein.	2 pages
Statement Deleting Inventors	[""] pages
Sequence Statement	[""] pages
Sequence Listing on Paper	[""] pages
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Small Entity Statement, which is: <input type="checkbox"/> Unsigned; <input type="checkbox"/> Newly signed for this application; <input type="checkbox"/> A copy from prior application [**SERIAL NUMBER**] and such small entity status is still proper and desired.	[**] pages
Preliminary Amendment	[**] pages
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APPLICATION

FOR

UNITED STATES LETTERS PATENT

APPLICANTS : HAI-YING ZHU, DENNIS GONSALVES, AND
KAI-SHU LING

TITLE : GRAPEVINE LEAFROLL VIRUS (TYPE 2)
PROTEINS AND THEIR USES

00140-9421960

GRAPEVINE LEAFROLL VIRUS (TYPE 2) PROTEINS AND THEIR USES

This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/047,194, filed May 20, 1997. This work was supported by the U.S. Department of Agriculture Cooperative Grant No. 58-2349-9-01. The U.S. Government may have certain rights in the invention.

FIELD OF THE INVENTION

The present invention relates to grapevine leafroll virus (type 2) proteins, DNA molecules encoding these proteins, and their uses.

BACKGROUND OF THE INVENTION

The world's most widely grown fruit crop, the grape (*Vitis sp.*), is cultivated on all continents except Antarctica. However, major grape production centers are in European countries (including Italy, Spain, and France), which constitute about 70% of the world grape production (Mullins et al., Biology of the Grapevine, Cambridge, U.K.:University Press (1992)). The United States, with 300,000 hectares of grapevines, is the eighth largest grape grower in the world. Although grapes have many uses, a major portion of grape production (~80%) is used for wine production. Unlike cereal crops, most of the world's vineyards are planted with traditional grapevine cultivars, which have been perpetuated for centuries by vegetative propagation. Several important grapevine virus and virus-like diseases, such as grapevine leafroll, corky bark, and *Rupestris* stem pitting, are transmitted and spread through the use of infected vegetatively propagated materials. Thus, propagation of certified, virus-free materials is one of the most important disease control measures. Traditional breeding for disease resistance is difficult due to the highly heterozygous nature and outcrossing behavior of grapevines, and due to polygenic patterns of inheritance. Moreover, introduction of a new cultivar may be prohibited by custom or law. Recent biotechnology developments have made possible the introduction of special traits, such as disease resistance, into an established cultivar without altering its horticultural characteristics.

Many plant pathogens, such as fungi, bacteria, phytoplasmas, viruses, and nematodes can infect grapes, and the resultant diseases can cause substantial losses in production (Pearson et al., Compendium of Grape Diseases, American Phytopathological

Society Press (1988)). Among these, viral diseases constitute a major hindrance to profitable growing of grapevines. About 34 viruses have been isolated and characterized from grapevines. The major virus diseases are grouped into: (1) the grapevine degeneration caused by the fanleaf nepovirus, other European nepoviruses, and American nepoviruses, (2) the leafroll complex, and (3) the rugose wood complex (Martelli, ed., Graft Transmissible Diseases of Grapevines. Handbook for Detection and Diagnosis, FAO, UN, Rome, Italy (1993)).

Of the major virus diseases, the grapevine leafroll complex is the most widely distributed throughout the world. According to Goheen ("Grape Leafroll," in Frazier et al., eds., Virus Diseases of Small Fruits and Grapevines (A Handbook), University of California, Division of Agricultural Sciences, Berkeley, Calif, USA, pp. 209-212 (1970) ("Goheen (1970)"), grapevine leafroll-like disease was described as early as the 1850s in German and French literature. However, the viral nature of the disease was first demonstrated by Scheu (Scheu, "Die Rollkrankheit des Rebstockes (Leafroll of grapevine)," D. D. Weinbau 14:222-358 (1935) ("Scheu (1935)"). In 1946, Harmon and Snyder (Harmon et al., "Investigations on the Occurrence, Transmission, Spread and Effect of 'White' Fruit Colour in the Emperor Grape," Proc. Am. Soc. Hort. Sci. 74:190-194 (1946)) determined the viral nature of White Emperor disease in California. It was later proven by Goheen et al. (Goheen et al., "Leafroll (White Emperor Disease) of Grapes in California," Phytopathology, 48:51-54 (1958) ("Goheen (1958)")) that both leafroll and "White Emperor" diseases were the same, and only the name "leafroll" was retained.

Leafroll is a serious viral disease of grapes and occurs wherever grapes are grown. This wide distribution of the disease has come about through the propagation of diseased vines. It affects almost all cultivated and wild varieties of *Vitis*. Although the disease is not lethal, it causes yield losses and reduction of sugar content. Scheu estimated in 1936 that 80 per cent of all grapevines planted in Germany were infected (Scheu, Mein Winzerbuch, Berlin:Reichsnährstand-Verlags (1936)). In many California wine grape vineyards, the incidence of leafroll (based on a survey of field symptoms conducted in 1959) agrees with Scheu's initial observation in German vineyards (Goheen et al., "Studies of Grape Leafroll in California," Amer. J. Enol. Vitic., 10:78-84 (1959)). The current situation on leafroll disease does not seem to be any better (Goheen, "Diseases Caused by Viruses and Viruslike Agents," The American Phytopathological Society, St. Paul, Minnesota:APS Press, 1:47-54 (1988) ("Goheen (1988)"). Goheen also estimated that the disease causes an annual loss of about 5-20 per cent of the total grape production (Goheen (1970) and Goheen (1988)).

The amount of sugar in individual berries of infected vines is only about 1/2 to 2/3 that of berries from noninfected vines (Goheen (1958)).

Symptoms of leafroll disease vary considerably depending upon the cultivar, environment, and time of the year. On red or dark-colored fruit varieties, the typical downward rolling and interveinal reddening of basal, mature leaves is the most prevalent in autumn; but not in spring or early summer. On light-colored fruit varieties however, symptoms are less conspicuous, usually with downward rolling accompanied by interveinal chlorosis. Moreover, many infected rootstock cultivars do not develop symptoms. In these cases, the disease is usually diagnosed with a woody indicator indexing assay using *Vitis* *vitifera* cv. Carbernet Franc (Goheen (1988)).

Ever since Scheu demonstrated that leafroll was graft transmissible, a virus etiology has been suspected (Scheu (1935)). Several virus particle types have been isolated from leafroll diseased vines. These include potyvirus-like (Tanne et al., "Purification and Characterization of a Virus Associated with the Grapevine Leafroll Disease," Phytopathology, 67:442-447 (1977)), isometric virus-like (Castellano et al., "Virus-like Particles and Ultrastructural Modifications in the Phloem of Leafroll-affected Grapevines," Vitis, 22:23-39 (1983) ("Castellano (1983)")) and Namba et al., "A Small Spherical Virus Associated with the Ajinashika Disease of Koshu Grapevine, Ann. Phytopathol. Soc. Japan, 45:70-73 (1979)), and closterovirus-like (Namba, "Grapevine Leafroll Virus, a Possible Member of Closteroviruses, Ann. Phytopathol. Soc. Japan, 45:497-502 (1979)) particles. In recent years, however, long flexuous closteroviruses ranging from 1,400 to 2,200 nm have been most consistently associated with leafroll disease (Figure 1) (Castellano (1983), Faoro et al., "Association of a Possible Closterovirus with Grapevine Leafroll in Northern Italy," Riv. Patol. Veg., Ser IV, 17:183-189 (1981), Gugerli et al., "L'enroulement de la vigne: mise en évidence de particules virales et développement d'une méthode immuno-enzymatique pour le diagnostic rapide (Grapevine Leafroll: Presence of Virus Particles and Development of an Immuno-enzyme method for Diagnosis and Detection)," Rev. Suisse Viticult. Arboricult. Hort., 16:299-304 (1984) ("Gugerli (1984)"), Hu et al., "Characterization of Closterovirus-like Particles Associated with Grapevine Leafroll Disease," J. Phytopathol., 128:1-14 (1990) ("Hu (1990)"), Milne et al., "Closterovirus-like Particles of Two Types Associated with Diseased Grapevines," Phytopathol. Z., 110:360-368 (1984), Zee et al., "Cytopathology of Leafroll-diseased Grapevines and the Purification and Serology of Associated Closteroviruslike Particles," Phytopathology, 77:1427-1434 (1987) ("Zee (1987)"), and Zimmermann et al., "Characterization and Serological Detection of Four

Closterovirus-like Particles Associated with Leafroll Disease on Grapevine," J. Phytopathol., 130:205-218 (1990) ("Zimmermann (1990)"). These closteroviruses are referred to as grapevine leafroll associated viruses ("GLRaV"). At least six serologically distinct types of GLRaV's (GLRaV-1 to -6) have been detected from leafroll diseased vines (Table 1) (Boscia et al., "Nomenclature of Grapevine Leafroll-associated Putative Closteroviruses, Vitis, 34:171-175 (1995) ("Boscia (1995)") and (Martelli, "Leafroll," pp. 37-44 in Martelli, ed., Graft Transmissible Diseases of Grapevines, Handbook for Detection and Diagnosis, FAO, Rome Italy, (1993) ("Martelli I"). The first five of these were confirmed in the 10th Meeting of the International Council for the Study of Virus and Virus Diseases of the Grapevine ("ICVG") (Volos, Greece, 1990).

TABLE 1

Type	Particle length (nm)	Coat protein <i>Mr</i> (X10 ³)	Reference
GLRaV-1	1,400-2,200	39	Gugerli (1984)
GLRaV-2	1,400-1,800	26	Gugerli (1984) Zimmermann (1990)
GLRaV-3	1,400-2,200	43	Zee (1987)
GLRaV-4	1,400-2,200	36	Hu (1990)
GLRaV-5	1,400-2,200	36	Zimmermann (1990)
GLRaV-6	1,400-2,200	36	Gugerli (1993)

- 15 Through the use of monoclonal antibodies, however, the original GLRaV II described in Gugerli (1984) has been shown to be an apparent mixture of at least two components, IIa and IIb (Gugerli et al., "Grapevine Leafroll Associated Virus II Analyzed by Monoclonal Antibodies," 11th Meeting of the International Council for the Study of Viruses and Virus Diseases of the Grapevine, Montreux, Switzerland, pp. 23-24 (1993) ("Gugerli (1993)").
- 20 Recent investigation with comparative serological assays (Boscia (1995)) demonstrated that the IIb component of cv. Chasselas 8/22 is the same as the GLRaV-2 isolate from France (Zimmermann (1990)) which also include the isolates of grapevine corky bark associated closteroviruses from Italy (GCBaV-BA) (Boscia (1995)) and from the United States

(GCBaV-NY) (Namba et al., "Purification and Properties of Closterovirus-like Particles Associated with Grapevine Corky Bark Disease," Phytopathology, 81:964-970 (1991) ("Namba (1991)"). The IIA component of cv. Chasselas 8/22 was given the provisional name of grapevine leafroll associated virus 6 (GLRaV-6). Furthermore, the antiserum to the CA-5 isolate of GLRaV-2 produced by Boscia et al. (Boscia et al., "Characterization of Grape Leafroll Associated Closterovirus (GLRaV) Serotype II and Comparison with GLRaV Serotype III," Phytopathology, 80:117 (1990)) was shown to contain antibodies to both GLRaV-2 and GLRaV-1, with a prevalence of the latter (Boscia (1995)).

Virions of GLRaV-2 are flexuous, filamentous particles about 1,400-1,800 nm in length (Gugerli et al., "L'enroulement de la Vigne: Mise en Evidence de Particules Virales et Development d'une Methode Immuno-enzymatique Pour le Diagnostic Rapide (Grapevine Leafroll: Presence of Virus Particles and Development of an Immuno-enzyme Method for Diagnosis and Detection)," Rev. Suisse Viticult. Arboricult. Hortic., 16:299-304 (1984)). A double-stranded RNA (dsRNA) of about 15 kb was consistently isolated from GLRaV-2 infected tissues (Goszczynski et al., "Detection of Two Strains of Grapevine Leafroll-Associated Virus 2," Vitis 35:133-35 (1996)). The coat protein of GLRaV-2 is ca 22-26 kDa (Zimmermann et al., "Characterization and Serological Detection of Four Closterovirus-like Particles Associated with Leafroll Disease on Grapevine," J. Phytopathology 130:205-18 (1990); Gugerli and Ramel, Extended abstracts: "Grapevine Leafroll Associated Virus II Analyzed by Monoclonal Antibodies," 11th ICVG at Montreux, Switzerland, Gugerli, ed., Federal Agricultural Research Station of Changins, CH-1260 Nyon, Switzerland, p. 23-24 (1993); Boscia et al., "Nomenclature of Grapevine Leafroll-Associated Putative Closteroviruses," Vitis 34:171-75 (1995)), which is considerably smaller than other GLRaVs (35-43 kDa) (Zee et al., "Cytopathology of Leafroll-Diseased Grapevines and the Purification and Serology of Associated Closterovirus Like Particles," Phytopathology 77:1427-34 (1987); Hu et al., "Characterization of Closterovirus-Like Particles Associated with Grapevine Leafroll Disease," J. of Phytopathology 128:1-14 (1990); Ling et al., "The Coat Protein Gene of Grapevine Leafroll Associated Closterovirus-3: Cloning, Nucleotide Sequencing and Expression in Transgenic Plants," Arch. of Virology 142:1101-16 (1997)). Although GLRaV-2 has been classified as a member of the genus *Closterovirus* based on particle morphology and cytopathology (Martelli, Circular of ICTV-Plant Virus Subcommittee Study Group on Closterolike Viruses" (1996)), its molecular and biochemical properties are not well characterized.

In the closterovirus group, several viruses have recently been sequenced. The partial or complete genome sequences of beet yellows virus (BYV) (Agranovsky et al.

"Nucleotide Sequence of the 3'-Terminal Half of Beet Yellows Closterovirus RNA Genome Unique Arrangement of Eight Virus Genes," J. General Virology 72:15-24 (1991);

- 5 Agranovsky et al., "Beet Yellows Closterovirus: Complete Genome Structure and Identification of a Papain-like Thiol Protease," Virology 198:311-24 (1994)), beet yellow stunt virus (BYSV) (Karasev et al., "Organization of the 3'-Terminal Half of Beet Yellow Stunt Virus Genome and Implications for the Evolution of Closteroviruses," Virology 221:199-207 (1996)), citrus tristeza virus (CTV) (Pappas et al., "Nucleotide Sequence and
- 10 Organization of Eight 3' Open Reading Frames of the Citrus Tristeza Closterovirus Genome," Virology 199:35-46 (1994); Karasev et al., "Complete Sequence of the Citrus Tristeza Virus RNA Genome," Virology 208:511-20 (1995)), lettuce infectious yellows virus (LIYV) (Klaassen et al., "Partial Characterization of the Lettuce Infectious Yellows Virus Genomic RNAs, Identification of the Coat Protein Gene and Comparison of its Amino Acid Sequence
- 15 With Those of Other Filamentous RNA Plant Viruses," J. General Virology 75:1525-33 (1994); Klaassen et al., "Genome Structure and Phylogenetic Analysis of Lettuce Infectious Yellows Virus, a Whitefly-Transmitted, Bipartite Closterovirus," Virology 208:99-110 (1995)), little cherry virus (LChV) (Keim and Jelkmann, "Genome Analysis of the 3'-Terminal Part of the Little Cherry Disease Associated dsRNA Reveals a Monopartite
- 20 Clostero-Like Virus," Arch. Virology 141:1437-51 (1996); Jelkmann et al., "Complete Genome Structure and Phylogenetic Analysis of Little Cherry Virus, a Mealybug-Transmissible Closterovirus," J. General Virology 78:2067-71 (1997)), and GLRaV-3 (Ling et al., "Nucleotide Sequence of the 3' Terminal Two-Thirds of the Grapevine Leafroll Associated Virus-3 Genomic RNA Reveals a Typical Monopartite Closterovirus," J. Gen. Virology
- 25 79(5):1289-1301 (1998)) revealed several common features of the closteroviruses, including the presence of HSP70 chaperone heat shock protein and a duplicate of the coat protein gene (Agranovsky "Principles of Molecular Organization, Expression, and Evolution of Closteroviruses: Over the Barriers," Adv. in Virus Res. 47:119-218 (1996); Dolja et al.
- 30 "Molecular Biology and Evolution of Closteroviruses: Sophisticated Build-up of Large RNA Genomes," Annual Rev. Photopathology 32:261-85 (1994); Boyko et al., "Coat Protein Gene Duplication in a Filamentous RNA Virus of Plants," Proc. Nat. Acad. Sci. USA 89:9156-60 (1992)). Characterization of the genome organization of GLRaVs would provide molecular information on the serologically distinct closteroviruses that cause similar leafroll symptoms in grapevine.

Several shorter closteroviruses (particle length 800 nm long) have also been isolated from grapevines. One of these, called grapevine virus A ("GVA") has also been found associated, though inconsistently, with the leafroll disease (Agran et al., "Occurrence of Grapevine Virus A (GVA) and Other Closteroviruses in Tunisian Grapevines Affected by Leafroll Disease," Vitis, 29:43-48 (1990), Conti, et al., "Closterovirus Associated with Leafroll and Stem Pitting in Grapevine," Phytopathol. Mediterr., 24:110-113 (1985), and Conti et al., "A Closterovirus from a Stem-pitting-diseased Grapevine," Phytopathology, 70:394-399 (1980)). The etiology of GVA is not really known; however, it appears to be more consistently associated with rugose wood *serius lato* (Rosciglione et al., "Maladies de l'enroulement et du bois strié de la vigne: analyse microscopique et sérologique (Leafroll and Stem Pitting of Grapevine: Microscopical and Serological Analysis)," Rev. Suisse Vitic Arboric. Horti., 18:207-211 (1986) ("Rosciglione (1986)"), and Zimmermann (1990)). Moreover, another short closterovirus (800 nm long) named grapevine virus B ("GVB") has been isolated and characterized from corky bark-affected vines (Boscia et al., "Properties of a Filamentous Virus Isolated from Grapevines Affected by Corky Bark," Arch. Virol., 130:109-120 (1993) and Namba (1991)).

As suggested by Martelli I, leafroll symptoms may be induced by more than one virus or they may be simply a general plant physiological response to invasion by an array of phloem-inhabiting viruses. Evidence accumulated in the last 15 years strongly favors the idea that grapevine leafroll is induced by one (or a complex) of long closteroviruses (particle length 1,400 to 2,200 nm).

Grapevine leafroll is transmitted primarily by contaminated scions and rootstocks. However, under field conditions, several species of mealybugs have been shown to be the vector of leafroll (Engelbrecht et al., "Transmission of Grapevine Leafroll Disease and Associated Closteroviruses by the Vine Mealybug Planococcus-ficus," Phytophylactica, 22:341-346 (1990), Rosciglione, et al., "Transmission of Grapevine Leafroll Disease and an Associated Closterovirus to Healthy Grapevine by the Mealybug Planococcus ficus," (Abstract), Phytoparasitica, 17:63-63 (1989), and Tanne, "Evidence for the Transmission by Mealybugs to Healthy Grapevines of a Closter-like Particle Associated with Grapevine Leafroll Disease," Phytoparasitica, 16:288 (1988)). Natural spread of leafroll by insect vectors is rapid in various parts of the world. In New Zealand, observations of three vineyards showed that the number of infected vines nearly doubled in a single year (Jordan et al., "Spread of Grapevine Leafroll and its Associated Virus in New Zealand Vineyards," 11th Meeting of the International Council for the Study of Viruses and Virus Diseases of the

Grapevine, Montreux, Switzerland, pp. 113-114 (1993)). One vineyard became 90% infected 5 years after GLRaV-3 was first observed. Prevalence of leafroll worldwide may increase as chemical control of mealybugs becomes more difficult due to the unavailability of effective insecticides.

In view of the serious risk grapevine leafroll virus poses to vineyards and the absence of an effective treatment of it, the need to prevent this affliction continues to exist. The present invention is directed to overcoming this deficiency in the art.

SUMMARY OF INVENTION

The present invention relates to an isolated protein or polypeptide corresponding to a protein or polypeptide of a grapevine leafroll virus (type 2). The encoding RNA and DNA molecules, in either isolated form or incorporated in an expression system, a host cell, a transgenic *Vitis* or *citrus* scion or rootstock cultivar, or a transgenic *Nicotiana* plant or beet plant are also disclosed.

Another aspect of the present invention relates to a method of imparting grapevine leafroll virus (type 2) resistance to *Vitis* scion or rootstock cultivars or *Nicotiana* plants by transforming them with a DNA molecule encoding the protein or polypeptide corresponding to a protein or polypeptide of a grapevine leafroll virus (type 2). Other aspects of the present invention relate to a method of imparting beet yellows virus resistance to beet plants and a method of imparting tristeza virus resistance to citrus scion or rootstock cultivars, both by transforming the plants or cultivars with a DNA molecule encoding the protein or polypeptide corresponding to a protein or polypeptide of a grapevine leafroll virus (type 2).

The present invention also relates to an antibody or binding portion thereof or probe which recognizes the protein or polypeptide.

Grapevine leafroll virus resistant transgenic variants of the current commercial grape cultivars and rootstocks allows for more complete control of the virus, while retaining the varietal characteristics of specific cultivars. Furthermore, these variants permit control of GLRaV-2 transmitted either by contaminated scions or rootstocks or by a presently uncharacterized insect vector. With respect to the latter mode of transmission, the present invention circumvents increased restriction of pesticide use which has made chemical control of insect infestation increasingly difficult. In this manner, the interests of the environment

and the economics of grape cultivation and wine making are all furthered by the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

5

Figures 1A and 1B are a comparison of a double-stranded RNA (dsRNA) profile (Figure 1A) of GLRaV-2 and its Northern hybridization analysis (Figure 1B). In Figure 1A: lane M, lambda Hind III DNA marker; and lane 1, dsRNA pattern in 1% agarose gel stained with ethidium bromide. Figure 1B is a northern hybridization of isolated high molecular weight dsRNA of GLRaV-2 with a probe prepared with ³²P [α -dATP] labeled cDNA insert from GLRaV-2 specific cDNA clone TC-1. Lane 1, high molecular weight dsRNA of GLRaV-2. Lane 2, total RNA extracted from healthy grapevine.

Figure 2 displays the genome organization of GLRaV-2 and its sequencing strategy. Boxes represent ORFs encoded by deduced amino acid sequences of GLRaV-2, numbered lines represent nucleotide coordinates, beginning from 5'-terminal of RNA in kilobases (kb). The lines below GLRaV-2 RNA genome represent the cDNA clones used to determine the nucleotide sequences.

Figure 3A-3D are comparisons between ORF1a/ORF1b of GLRaV-2 and BYV. Figure 3A-3D show the conserved domains of two papain-like proteases (P-PRO), methyltransferase (MT/MTR), helicase (HEL), and RNA-dependent RNA polymerase (RdRP), respectively. Exclamation marks indicate the predicted catalytic residues of the leader papain-like protease; slashes indicate the predicted cleavage sites. The conserved motifs of the MT, HEL, and RdRP domains are highlighted with overlines marked with respective letters. The alignment is constructed using the MegAlign program in DNASTAR.

Figures 4A and 4B are alignments of the nucleotide (Figure 4A) and deduced amino acid (Figure 4B) sequences of ORF1a/ORF1b overlapping region of GLRaV-2, BYSV, and CTV. Identical nucleotides and amino acids are shown in consensus. GLRaV-2 putative + 1 frameshift site (TAGC) and its corresponding sites of BYV (TAGC) and BYSV (TAGC) and CTV (CGGC) at nucleotide and amino acid sequences are highlighted with underlines.

Figure 5 is an alignment of the amino acid sequence of HSP70 protein of GLRaV-2 and BYV. The conserved motifs (A to H) are indicated with overlines and marked

with respective letters. The alignment was conducted with the MegAlign program of DNASTAR.

Figure 6A is a comparison of the coat protein (CP) and coat protein duplicate (CPd) of GLRaV-2 with other closteroviruses. The amino acid sequence of the GLRaV-2 CP and CPd are aligned with the CP and CPd of BYV, BYSV, and CTV. The conserved amino acid residues are in bold and the consensus sequences are indicated. Sequence alignment and phylogenetic tree were constructed by Clustal Method in the MegAlign Program of DNASTAR. Figure 6B is a tentative phylogenetic tree of the CP and CPd of GLRaV-2 with BYV, BYSV, CTV, LIYV, LChV, and GLRaV-3. To facilitate the alignment, only the C-terminal 250 amino acids of CP and CPd of LIYV, LChV, and GLRaV-3 were used. The scale beneath the phylogenetic tree represents the distance between sequences. Units indicate the number of substitution events.

Figure 7 is a comparison of the genome organization of GLRaV-2, BYV, BYSV, CTV, LIYV, LChV, and GLRaV-3. P-PRO, papain-like protease; MT/MTR, methyltransferase; HEL, helicase; RdRP, RNA-dependent RNA polymerase; HSP70, heat shock protein 70; CP, coat protein; CPd, coat protein duplicate.

Figure 8 is a tentative phylogenetic tree showing the relationship of RdRP of GLRaV-2 with respect to BYV, BYSV, CTV, and LIYV. The phylogenetic tree was constructed using the Clustal method with the MegAlign program in DNASTAR.

Figure 9 is an alignment of the amino acid sequence of HSP90 protein of GLRaV-2 with respect to other closteroviruses, BYV, BYSV, and CTV. The most conserved motifs (I to II) are indicated with the highlighted lines and marked with respective letters.

Figure 10 is an alignment of the nucleotide sequence of 3'-terminal untranslated region of GLRaV-2 with respect to the closteroviruses BYV (Agranovsky et al., "Beet Yellows Closterovirus: Complete Genome Structure and Identification of a Papain-like Thiol Protease," Virology 198:311-24 (1994), which is hereby incorporated by reference), BYSV (Karasev et al., Organization of the 3'-Terminal Half of Beet Yellow Stunt Virus Genome and Implications for the Evolution of Closteroviruses," Virology 221:199-207 (1996), which is hereby incorporated by reference), and CTV (Karasev et al., "Complete Sequence of the Citrus Tristeza Virus RNA Genome," Virology 208:511-20 (1995), which is hereby incorporated by reference). The consensus sequences are shown, and the distance to the 3'-end is indicated. A complementary region capable of forming a "hair-pin" structure is underlined.

Figures 11A and 11B are genetic maps of the transformation vectors

pGA482GG/EPT8CP-GLRaV-2 and pGA482G/EPT8CP-GLRaV-2, respectively. As shown in Figures 11A and 11B, the plant expression cassette (EPT8CP-GLRaV-2), which consists of a double cauliflower mosaic virus (CaMV) 35S-enhancer, a CaMV 35S-promoter, an alfalfa mosaic virus (ALMV) RNA4 5' leader sequence, a coat protein gene of GLRaV-2 (CP-GLRaV-2), and a CaMV 35S 3' untranslated region as a terminator, was cloned into the transformation vector by EcoR I restriction site. The CP of GLRaV-2 was cloned into the plant expression vector by Nco I restriction site.

Figure 12 is a PCR analysis of DNA molecules extracted from the leaves of putative transgenic plants using both the CP gene of GLRaV-2 and NPT II gene specific primers. An ethidium bromide-stained gel shows a 720 bp amplified DNA fragment for NPT II gene, and a 653 bp DNA fragment for the entire coding sequence of the CP gene. Lane 1, Φ 174 / Hae III DNA Marker; lanes 2-6, transgenic plants from different lines; lane 7, the cp gene of GLRaV-2 of positive control; and lane 8, NPT II gene of positive control.

Figure 13 is a comparison of resistant (right side 3 plants) and susceptible (left side 3 plants) transgenic *Nicotiana benthamiana* plants. Plants are shown 48 days after inoculation with GLRaV-2.

Figure 14 is a northern blot analysis of transgenic *Nicotiana benthamiana* plants. An aliquot of 10 g of total RNA extracted from putative transgenic plants was denatured and loaded onto 1% agarose gel containing formaldehyde. The separated RNAs were transferred to Gene Screen Plus membrane and hybridized with a 32 P-labeled DNA probe containing the 3' one third CP gene sequence. Lanes 1, 3, and 4 represent nontransformed control plants without RNA expression. The remaining lanes represent transgenic plants from different lines: lanes 2, 14-17, and 22-27 represent plants with high RNA expression level which are susceptible to GLRaV-2; all other lanes represent plants with undetectable or low RNA expression level which are resistant to GLRaV-2.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to isolated DNA molecules encoding for the proteins or polypeptides of a grapevine leafroll virus (type 2). A substantial portion of the grapevine leafroll virus (type-2) ("GLRaV-2") genome has been sequenced. Within the genome are a plurality of open reading frames ("ORFs") and a 3' untranslated region ("UTR"), each containing DNA molecules in accordance with the present invention. The

DNA molecule which constitutes a substantial portion of the GLRaV-2 genome comprises the nucleotide sequence corresponding to SEQ. ID. No. 1 as follows:

1451

TAAACATTGC GAGAGAACCC CATTAGCGTC TCCGGGGTGA ACTTGGGAAG GTCTGCCGCC 60

GCTCAGGTTA TTTATTTCGG CAGTTTCACG CAGCCCTTCG CGTTGTATCC GCGCCAAGAG 120

AGCGCGATCG TAAAAACGCA ACTTCCACCG GTCAGTGTAG TGAAGGTGGA GTGCGTAGCT 180

GCGGAGGTAG CTCGCCGACAG GGGCGTGGTC GACAAGAAAC CTACGTCGTG TGGCGTTCCC 240

CCGCAGCGCG GTGTGCTTTC TTTTCCGACG GTGGTTCGGA ACCGCGGCGA CGTGATAATC 300

ACAGGGGTGG TGCATGAAGC CCTGAAGAAA ATTAAAGACG GGCTCTTACG CTTCCGCGTA 360

GGCGGTGACA TGC GTTTTTT CAGATTTTTC TCATCGAACT ACGGCTGCGA ATTCGTGCGG 420

AGCGTGCCTA CGAACACTAC AGTTTGGCTA AATTGCACGA AAGCGAGTGG TGAGAAATTC 480

TCACTCGCCG CCGCGTGCAC GCGGATTAC GTGGCGATGC TGC GTTATGT GTGTGGCGGG 540

AAATTTCCAC TCGTCTCAT GAGTAGAGTT ATTTACCCGG ATGGCGCGTG TTA CTGGCC 600

CATATGAGGT ATTTGTGCGC CTTTACTGT CGCCCGTTTA GAGAGTCGGA TTATGCCCTC 660

GGAATGTGGC CTACGTTGGC GCGTCTCAGG GCATGCGTTG AGAAGAAGCT CGGTGTCGAA 720

GCTGTGGCA TAGCTCTTCG TGCTATTAC ACCTCTCGCA ATGTTTATCA CTGTGATTAT 780

GACTCTGCTT ATGTAAATA TTTTAGAAAC CTTTCCGGCC GCATTGGCGG TGGTTCGTTT 840

GATCCGACAT CTTTAACTC CGTAATAACG GTGAAGATTA GCGGTCTTCC AGGTGGCTTT 900

CCTAAAAATA TAGCGTTTGG TGCTTCTCG TGCGATATAC GTTACGTCGA ACCGGTAGAC 960

TCGGGCGGCA TTCAATCGAG CGTTAAGACG AAACGTGAAG ATGCGCACCG AACCGTAGAG 1020

GAACGGGCGG CCGGCGGATC CGTCGAGCAA CCGCGACAAA AGAGGATAGA TGAGAAAGGT 1080

TGCGGCAGAG TTCTAGTGG AGGTTTTTCG CATCTCCTGG TCGGCAACCT TAACGAAGTT 1140

AGGAGGAAG TAEGTCCCGG ACTTCTACGC TTTCGCGTTG GCGGTGATAT GGATTTTCAT 1200

CGCTCGTTCT CCACCAAGC GGGCCACCGC TTGCTGGTGT GGGCCGCGTC GAGCCGGAGC 1260

GTGTGCCTTG AACTTTACTC ACCATCTAAA AACTTTTTCG GTTACGATGT CTGCCCCGTG 1320

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ATGACTAGAA TTAGATACCC GAACGGGTTT TGTTACTTGG CTCACTGCCG GTACGCGTGC 1440

GCGTTTCTCT TAAGGGGTTT TGATCCGAAG CGTTTCGACA TCGGTGCTTT CCCCACCGCG 1500

GCCAAGCTCA GAAACCGTAT GGTTCGGAG CTTGGTGAAA GAAGTTTAGG TTTGAACTTG 1560

TACGGCGCAT ATACGTCACG CGGCGTCTTT CACTGCGATT ATGACGCTAA GTTTATAAAG 1620

GATTTGCGTC TTATGTCAGC AGTTATAGCT GGAAAGGACG GGGTGAAGA GGTGTACCT 1680

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TCGACATAA	CTCCTGCCAT	GAAGCAGAAA	ACGATCGAAG	CCGTGTATGA	TAGATTATAT	1740
GGCGGCACTG	ACTCGTTGCT	GAAACTGAGC	ATCGAGAAAG	ACTTAATCGA	TTTCAAAAAT	1800
GACGTGCGA	GTTTGAAGAA	AGATCGGCCG	ATTGTCAAAG	TGCCCTTTTA	CATGTCGGAA	1860
GCAACACAGA	ATTCGCTGAC	GCGTTTCTAC	CCTCAGTTCG	AACTTAAGTT	TTCGCACCTC	1920
TCGCATTAG	ATCATCCCGC	CGCGCCGCT	TCTAGACTGC	TGGAAAATGA	AACGTTAGTG	1980
CGCTTATGTG	GTAATAGCGT	TTCAGATATT	GGAGGTTGTC	CTCTTTTCCA	TTTGCACTCC	2040
AAGACGCAAA	GACGGGTTCA	CGTATGTAGG	CCTGTGTTTG	ATGGCAAGGA	TGCGCAGCGT	2100
CGCGTGGTGC	GTGATTTGCA	GTATTCCAAC	GTGCGTTTGG	GAGACGATGA	TAAAATTTTG	2160
GAAGGGCCAC	GCAATATCGA	CATTTGCCAC	TATCCTCTGG	GCGCGTGTA	CCACGAAAGT	2220
AGTGCTATGA	TGATGGTGCA	GGTGTATGAC	GCGTCCCTTT	ATGAGATATG	TGGCGCCATG	2280
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TCGAAGTTTG	TGAGTCGCGT	TTTCGATTAT	GTGCTGTGTA	ATTGCTCTGC	CGTGAACTCA	2760
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GTTATGTTGG	CCTCTGGCGT	GCGCAGTAGA	CTAGCGTCCG	AGTACCTTGC	TAAGAACCTT	2940
AGTCATTTTT	CGGGAGATTG	CTCCTTTATT	GAAGGCGCTT	CTTTCGTGTT	GCGTGAGAAA	3000
ATCAGAAACA	TGACTCTGAA	TTTTAACGAA	AGACTTTTAC	AGTTAGTGAA	GCGCGTTGCC	3060
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AACGCCGATA	AGCGGGGAAGT	TCAGAGGCC	GGTTTGCGTG	GTGGTTCTAG	AAACGGGGTT	3360
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GAAGTGCTTA ACATTGAATG CCAGAAACGT AAAGACAAAA GATCTCCGAA AAAGAGCATT	6960
TACACCATCG ACGCTTATTT AATGCATCAC CGTGTTTGTG ATGCAGACGT TCTTTTCATC	7020
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CATAAAGTAA TGATCTTCGG GGATAGCCGG CAGATTCAC TACATTGAAAG GAACGAATTG	7140
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GCTTGGTGAC	GACGATGTGT	ACCATATAGG	TGAAGTTGAT	TTCTCAAAGT	ACGACAAGTC	8520
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AAAGTAGCAA TCCCCGTCTA TCGACAGCGC AACTATGGGT TGGTTTCTTT TGTTACTATG 12360
GTGAGTTTCG TACGGCTCAA AGTAGAGTAG TGCAAAGACC AGGCGTATAC AAAACACCTG 12420
ACTCAGTGGG TGGATTGAA ATAAACATGA AAGATGTTGA GAAATTCTTC GATAAACTTC 12480
AGAGAGAATT GCCTAATGTA TCTTTGCGGC GTCAGTTTAA CGGAGCTAGA GCGCATGAGG 12540
CTTTCAAAAT ATTTAAAAAC GGAATATATA GTTTCAGACC TATATCGCGT TTAACGTGC 12600
CTAGAGAGTT CTGGTATCTG AACATAGACT ACTTCAGGCA CGCGAATAGG TCCGGTTAA 12660

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CCGAAGAAGA AATACTCATC CTAAACAACA TAAGCGTTGA TGTAGGAAG TTATGCGCTG	12720
AGAGAGCGTG CAATACCCTA CCTAGCGCGA AGCGCTTTAG TAAAAATCAT AAGAGTAATA	12780
TACAATCATC ACGCCAAGAG CGGAGGATTA AAGACCCATT GGTAGTCTCG AAAGACACTT	12840
TATATGAGTT CCAACACAAG CGTGCCGTTT GGGGGTCTCG AAGCACTCGA GACCTCGGGA	12900
GTCGTGCTGA CCACGCGAAA GGAAGCGGTT GATAAGTTTT TTAATGAAC TAAAAACGAA	12960
AATTACTCAT CAGTTGACAG CAGCCGATTA ASCGATTCGG AAGTAAAGA AGTGTTAGAG	13020
AAAAGTAAAG AAGTTTCAA AAGCGAAGTG GCCTCCACTG ACGAGCACTT CGTCTACCAC	13080
ATTATATTTT TCTTAATCCG ATGTGCTAAG ATATCGACAA GTGAAAAGGT GAAGTACGTT	13140
GGTAGTCATA CGTACGTGTT CGACGGAAGA ACGTACACCG TTCTTGACGC TTGGGTATTC	13200
AACATGATGA AAGTCTCAC GAAGAAGTAC AAACGAGTGA ATGTCCTGCG TGCCTTCTGT	13260
TGCGCGTGGC AAGATCTATA TCTAACCGTC GCACCAATAA TGTCAGAACG CTTTAAGACT	13320
AAAGCCGTAG GGATGAAGG TTTGCCTGTT GGAAAGGAAT ACTTAGGCGC CGACTTTCTT	13380
TCGGGAACTA GCAAAGTAT GAGCGATCAC GACAGGCGCG TCTCCACTGT TGCAGCGAAA	13440
AACGCTGTCG ATCGTAGCGC TTTCACGGTT GGGGAGAGAA AGATAGTTAG TTTGTATGAT	13500
CTAGGGAGGT ACTAAGCACG GTGTGCTATA GTGCGTGCTA TAATAATAAA CACTAGTGCT	13560
TAAGTCGCGC AGAAGAAAAA GCTATGGAGT TGATGTCCGA CAGCAACCTT AGCAACCTGG	13620
TGATAACCGA CGCCTCTAGT CTAATGGGTG TCGACAAGAA GCTTTTATCT GCTGAAGTTG	13680
AAAAATGTT GGTGCAGAAA GGGGCTCCTA ACGAGGGTAT AGAAGTGGTG TTCGCTCTAC	13740
TCCTTTACGC ACTCGCGGCA AGAACCACGT CTCTAAGGT TCAGCGCGCA GATTGAGCG	13800
TTATATTTTC AAATAGTTTC GGAGAGAGGA ATGTGTTAGT AACAGAGGT GACCTTAAGA	13860
AGGTACTCGA CGGTTGTGCG CCTCTACTA GGTCTACTAA TAACTTAGA ACGTTCGGTC	13920
GTACTTTCAAGT GAGGCTTAC GTTGACTTTT GTATCGCGTA TAAGCACAAA TTACCCCAAC	13980
TCAACGCCGC GGCAGGAATT GGGATTCCAG CTGAAGATTC GTACTTAGCT GCAGATTTTC	14040
TGGGTACTTG CCCGAAGCTC TCTGAATTAC AGCAAAGTAG GAAGATGTTT GCGAGTATGT	14100
ACGCTCTAAA AACTGAAGGT GGAGTGGTAA ATACACCAGT GAGCAATCTG CGTCAGCTAG	14160
GTAGAAGGGA AGTTATGTAA TGGAAGATTA CGAAGAAAAA TCCGAATCGC TCATACTGCT	14220
ACGCACGAAT CTGAACACTA TGCTTTTAGT GGTCAAGTCC GATGCTAGTG TAGAGCTGCC	14280
TAAACTACTA ATTTGCGGTT ACTTACGAGT GTCAGGACGT GGGGAGGTGA CGTGTTGCAA	14340
CCGTGAGGAA TTAACAAGAG ATTTTGAGGG CAATCATCAT ACGGTGATCC GTTCTAGAAT	14400
CATACAATAT GACAGCGAGT CTGCTTTTGA GGAATTCAAC AACTCTGATT GCSTAGTGAA	14460
GTTTTTCCTA GAGACTGGTA GTGCTTTTGT GTTTTTCTT CGAAGTGAAA CCAAAGGTAG	14520

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AGCGGTGCGA	CATTTCGCGA	CCTTCTTCGA	AGCTAACAAT	TTCTTCTTTG	GATCGCATTG	14580
CGGTACCATG	GAGTATTGTT	TGAAGCAGGT	ACTAACTGAA	ACTGAATCTA	TAATCGATTG	14640
TTTTTGCAG	AAAAGAAATC	GTTAAGATGA	GGGTTATAGT	GTCTCCTTAT	GAAGCTGAAG	14700
ACATTCTGAA	AAGATCGACT	GACATGTTAC	GAAACATAGA	CAGTGGGGTC	TTGAGCACTA	14760
AAGAATGTAT	CAAGGCATTC	TCGACGATAA	CGCGAGACCT	ACATTGTGCG	AAGGCTTCCT	14820
ACCACTGGGG	TGTTGACACT	GGGTTATATC	AGCGTAATTG	CGCTGAAAAA	CGTTTAATTG	14880
ACACGGTGGA	GTCAAACATA	CGGTTGGGCT	AACCTCTCGT	CGCTGAAAAA	GTGGCGGTTG	14940
ATTTTGTGTA	GGATGAACCA	AAAGAGCTAG	TAGCATTTCAT	CACGCGAAAG	TACGTGGAAC	15000
TCACGGGCGT	GGGAGTGAGA	GAAGCGGTGA	AGAGGGAAAT	GCCTCTCTTT	ACCAAAACAG	15060
TTTTAAATAA	AATGTCTTTG	GAAATGGCGT	TTTACATGTC	ACCACGAGCG	TGAAAAACG	15120
CTGAATGGTT	AGAACTAAAA	TTTTACCTTG	TGAAAATCTT	TAGAGATCTG	CTATTAGACG	15180
TGGAACGCT	CAACGAATTG	TGCGCCGAAG	ATGATGTTCA	CGTCGACAAA	GTAATGAGA	15240
ATGGGGACGA	AAATCAGCAC	CTCGAATCC	AAGACGAATG	TTAAACATTG	GTTAAGTTTA	15300
ACGAAAATGA	TTAGTAATAA	ATAAATCGAA	CGTGGGTGTA	TCTACCTGAC	GTATCAACTT	15360
AAGCTGTTAC	TGAGTAATTA	AACCAACAAG	TGTTGGTGTA	ATGTGTATGT	TGATGTAGAG	15420
AAAAATCCGT	TTGTAGAACG	GTGTTTTTCT	CTTCTTTTAT	TTTAAAAAAA	AAATAAAAAA	15480
AAAAAAAAAA	AAGCGCCGCG					15500

Another DNA molecule of the present invention (GLRaV-2 ORF1a) includes nucleotides 4-7923 of SEQ. ID. No. 1 and is believed to code for a large, grapevine leafroll virus polyprotein containing the conserved domains characteristic of two papain-like proteases, a methyltransferase, and a helicase. This DNA molecule comprises the nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

ACATTGCGAG	AGAACCCCAT	TAGCGTCTCC	GGGGTGAACT	TGSGAAGGTC	TGCCGCCGCT	60
CAGGTTATTT	ATTTTCGCGAG	TTTCACGCAG	CCCTTCGCGT	TGTATCCGCG	CCAAGAGAGC	120
GCGATCGTAA	AAACGCAACT	TCCACCGGTC	AGTGTAGTGA	AGGTGGAGTG	CGTAGCTGCG	180
GAGGTAGCTC	CCGACAGGGG	CGTGGTCGAC	AAGAAACCTA	CGTCTGTTGG	CGTCCCCCCG	240
CAGCGCGGTG	TGCTTTCTTT	TCCGACGGTG	GTTCGGAACC	GCGGCGAGCT	GATAATCACA	300
GGGGTGGTGC	ATGAAGCCCT	GAAGAAAAAT	AAAGACGGGC	TCTTACGCTT	CCGCGTAGGC	360
GGTGACATGC	GTTTTTCGAG	ATTTTCTCTA	TCGAACCTACG	GCTGCAGATT	CGTCGCGAGC	420
GTGCGTACGA	ACACTACAGT	TTGGCTAAAT	TGCACGAAAG	CGAGTGGTGA	GAAATCTCTA	480

CTCGCGCGCG	CGTGACACGG	GGATTACGTG	GCAGTGCTGC	GTTATGTGTG	TGGCGGGAAA	540
TTTCCACTCG	TCCTCATGAG	TAGAGTTATT	TACCCGGATG	GGCGCTGTTA	CTTGGCCCAT	600
ATGAGGTATT	TGTGCGCCTT	TTACTGTGCG	CCGTTTAGAG	AGTCGGATTA	TGCCCTCGGA	660
ATGTGGCCTA	CGGTGGCGCG	TCTCAGGGCA	TGCGTTGAGA	AGAACTTCGG	TGTCGAAGCT	720
TGTGGCATAG	CTCTTCGTGG	CTATTACACC	TCTCGCAATG	TTTATCACTG	TGATTATGAC	780
TCTGCTTATG	TAAAATATTT	TAGAAACCTT	TCCGGCCGCA	TTGGCGGTGG	TTCGTTCGAT	840
CCGACATCTT	TAACCTCCGT	AATAACGGTG	AAGATTAGCG	GTCTTCCAGG	TGCTCTTCCT	900
AAAAATATAG	CGTTTGGTGC	CTTCTGTGCG	GATATACGTT	AAGTCGAACC	GGTAGACTCG	960
GGCGGCATTG	AATCGAGCGT	TAAGACGAAA	CGTGAAGATG	CGCACCGAAC	CGTAGAGGAA	1020
CGGGCGGCCG	CGCGATCCGT	CGAGCAACCG	CGACAAAAGA	GGATAGATGA	GAAAGGTTGC	1080
GGCAGAGTTC	CTAGTGGAGG	TTTTTCGCAT	CTCTGTGTCG	GCAACCTTAA	CGAAGTTAGG	1140
AGGAAGGTAG	CTGCCGGAAT	TCTACGCTTT	CGCGTTGGCG	GTGATATGGA	TTTTCATCGC	1200
TCGTTCTCCA	CCCAAGCGGG	CCACCGCTTG	CTGTTGTGGC	GCCGCTCGAG	CCGGAGCGTG	1260
TGCCTTGAAC	TTTACTCACC	ATCTAAAAAG	TTTTTGC GTT	ACGATGTCTT	GCCCTGTTCT	1320
GGAGACTATG	CAGCGATGTT	TTCTTTCGCG	GCGGGCGGCC	GTTTCCCTTT	AGTTTGTATG	1380
ACTAGAATTA	GATACCCGAA	CGGGTTTGTG	TACTTGGCTC	ACTGCCGGTA	CGCGTGC GCG	1440
TTTCTCTTAA	GGGTTTGTGA	TCCGAAGCGT	TTTGACATCG	GTGCTTTCCC	CACCGCGGCC	1500
AAGCTCAGAA	ACCGTATGGT	TTTCGGAGCTT	GGTGAAGAA	GTTTAGGTTT	GAAC TTGTAC	1560
GGCGCATATA	CGTCACGCGG	CGTCTTTCAC	TGCGATTATG	ACGCTAAGTT	TATAAAGGAT	1620
TTGCGTCTTA	TGTCAGCAGT	TATAGCTGGA	AAGGACGGGG	TGGAAGAGGT	GGTACCTTCT	1680
GACATAACTC	CTGCCATGAA	GCAGAAAACG	ATCGAAGCCG	TGTATGATAG	ATTATATGGC	1740
GGCACTGACT	CGTTGCTGAA	ACTGAGCATC	GAGAAAGACT	TAATCGATTT	CAAAAATGAC	1800
GTGCAGAGTT	TGAAGAAAGA	TCGGCCGATT	GTCAAAGTGC	CCTTTTACAT	GTCCGAAGCA	1860
ACACAGAATT	CGCTGACGCG	TTTCTACCCT	CAGTTCGAAC	TTAAGTTTTC	GCACTCCTCG	1920
CATTGAGATC	ATCCCCGCCG	CGCCGCTTCT	AGACTGCTGG	AAAATGAAAC	GTAGTGCGCG	1980
TTATGTGGTA	ATAGCGTTTC	AGATATTGGA	GGTTGCTCCT	TTTTCCATTT	GCATTTCCAAG	2040
ACGCAAAGAC	GGGTTACAGT	ATGTAGGCCCT	GTGTTGGATG	GCAAGGATGC	GCAGCGTCGCG	2100
GTGGTGC GTG	ATTTGCAGTA	TTCCAACGTG	CGTTTGGGAG	ACGATGATAA	AATTTTGGAA	2160
GGGCCACGCA	ATATCGACAT	TTGCCACTAT	CCTCTGGGCG	CGTGTGACCA	CGAAAGTAGT	2220
GCTATGATGA	TGGTGCAGGT	GTATGACGCG	TCCCTTTATG	AGATATGTGG	CGCCATGATC	2280

AAGAAGAAAA	GCCGCATAAC	GTACTTAACC	ATGGTCACGC	CCGGCGAGTT	TCTTGACGGA	2340
CGCGAATGCG	TCTACATGGA	GTGCTTAGAC	TGTGAGATTG	AAGTTGATGT	GCACGCGGAC	2400
GTGCTAATGT	ACAAATTCGG	TAGTTCTTGC	TATTGCGACA	AGCTTTCAAT	CATCAAGGAC	2460
ATCATGACCA	CTCCGTACTT	GACACTAGGT	GGTTTTCTAT	TCAGCGTGGA	GATGTATGAG	2520
GTGCGTATGG	GCGTGAATTA	CTTCAAGATT	ACGAAGTCCG	AAGTATCGCC	TAGCATTAGC	2580
TGCACCAAGC	TCCTGAGATA	CCGAAGAGCT	AATAGTGACG	TGGTTAAAGT	TAAACTTCCA	2640
CGTTTCGATA	AGAAACGTCG	CATGTGTCTG	CCTGGGTATG	ACACCATATA	CCTAGATTCT	2700
AAGTTTGTGA	GTGCGGTTTT	CGATTATGTC	GTGTGTAATT	GCTCTGCCGT	GAACTCAAAA	2760
ACTTTCGAGT	GGGTGTGGAG	TTTCATTAA	TCTAGTAAGT	CGAGGGTGAT	TATTAGCGGT	2820
AAAATAATTC	ACAAGGATGT	GAATTTGGAC	CTCAAGTACG	TCGAGAGTTT	CGCCGCGGTT	2880
ATGTTGGCCT	CTGGCGTGGC	CAGTAGACTA	GCGTCCGAGT	ACCTTGCTAA	GAACCTTAGT	2940
CATTTTTTCG	GAGATTGCTC	CTTTATTGAA	GCCACGTCTT	TCGTGTTGCG	TGAGAAAAATC	3000
AGAAACATGA	CTCTGAATTT	TAACGAAAGA	CTTTTACAGT	TAGTGAAGCG	CGTTGCCTTT	3060
GCGACCTTGG	ACGTGAGTTT	TCTAGATTTA	GATTCAACTC	TTGAATCAAT	AACGTATTTT	3120
GCCGAGTGTA	AGGTAGCGAT	TGAACTCGAG	GAGTTGGGTT	GCTTGAGAGC	GGAGGCCGAG	3180
AATGAAAAAA	TCAGGAATCT	GGCGGGAGAT	TCGATTGCGG	CTAAACTCGC	GAGCGAGATA	3240
GTGGTCGATA	TTGACTCTAA	GCCTTCACCG	AAGCAGGTGG	GTAATTCGTC	ATCCGAAAAC	3300
GCCGATAAGC	GGGAAGTTCA	GAGGCCCGGT	TTGCGTGGTG	GTTCTAGAAA	CGGGGTGTTT	3360
GGGGAGTTCC	TTCACCTTCG	CGTGGATTCT	GCCTTGCGTC	TTTTCAAATA	CGCGACGGAT	3420
CAACAACGGA	TCAAGTCTTA	CGTGCCTTTC	TTGGAATCGG	CGGTCTCATT	CTTGGATTAC	3480
AACACGATA	ATCTATCGTT	TATACTGCGA	GTGCTTTCGG	AAGGTTATTC	GTGTATGTTC	3540
GCGTTTTTGG	GGAAATCGCG	GCTCTTATCT	AGTCGTGTCC	GTAGCGCGGT	GTGTGCTGTG	3600
AAAGAAGTTG	CTACCTCATG	CGCGAACGCG	AGCGTTTCTA	AAGCCAAGGT	TATGATTACC	3660
TTGCGAGCGG	CCGTGTGTGC	TATGATGTTT	AATAGCTGCG	GTTTTTCAGG	CGACGGTCGG	3720
GAGTATAAAT	CGTATATACA	TCGTTACACG	CAAGTATTGT	TTGACACTAT	CTTTTTTGAG	3780
GACAGCAGTT	ACCTACCCAT	AGAAGTCTTG	AGTTCGGCGA	TATGCGGTGC	TATCGTCACA	3840
CTTTTCTCCT	CGGGCTCGTC	CATAAGTTTA	AACGCCTTCT	TACTTCAAAAT	TACCAAAGGA	3900
TTCTCCCTAG	AGGTTGTCGT	CCGGAATGTT	GTGCGAGTCA	CGCATGGTTT	GAGCACCACA	3960
GCGACCGACG	GCGTCATACG	TGGGGTTTTT	TCCCAAATTT	TGTCTCACTT	ACTTGTGTTG	4020
AATACGGGTA	ATGTGGCTTA	CCAGTCAGCT	TTCATTGCCG	GGGTGTTGCC	TCTTTTAGTT	4080
AAAAAGTGTG	TGAGCTTAAT	CTTCATCTTG	CGTGAAGATA	CTTATTCGGG	TTTTATTAA	4140

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CACGGAATCA GTGAATTCTC TTTCCTTAGT AGTATTCTGA AGTCTCTGAA GGGTAAGCTT	4200
GTGGACGAGT TGAATTCGAT TATTC AAGG GTTTTTGATT CCAACAAGCA CGTGTTTAAA	4260
GAAGCTACTC AGGAAGCGAT TCGTACGACG GTCATGCAAG TGCTGTGCGC TGTAGTGGAT	4320
GCCCTTAAAG GCGCCGCGGG AAAAATTTAT AACAAATTTA CTAGTCGACG TACCTTTGGT	4380
AAGGATGAAG GCTCCTCTAG CGACGCGCA TGTGAAGAGT ATTTCTCATG CGACGAAGGT	4440
GAAGGTCGGG GTCTGAAAGG GGGTTCAGC TATGGCTTCT CAATTTTAGC GTTCTTTTCA	4500
CGCATTATGT GGGGAGCTCG TCGGCTTATT GTTAAGGTGA AGCATGAGTG TTTTGGGAAA	4560
CTTTTGAAT TTCTATCGCT CAAGCTTCAC GAATTCAGGA CTCGCGTTTT TGGGAAGAAT	4620
AGAACGAGC TGGGAGTTTA CGATTTTTTG CCCACGGGCA TCGTGGAAAC GCTCTCATCG	4680
ATAGAAGAGT GCGACCAAAT TGAAGAACTT CTCGGCGACG ACCTGAAAGG TGACAAGGAT	4740
GCTTCGTTGA CCGATATGAA TTACTTTGAG TTCTCAGAAG ACTTCTTAGC CTCTATCGAG	4800
GAGCCGCCCT TCGCTGGATT GCGAGGAGGT AGCAAGAACA TCGCGATTTT GGCGATTTTG	4860
GAATACGCGC ATAATTTGTT TCGCATTGTC GCAAGCAAGT GTTCGAAACG ACCTTTATTT	4920
CTTGCTTTG CCGAACTCTC AAGCGCCCTT ATCGAAGAAAT TTAAGGAGGT TTTCCCTCGT	4980
AAGAGCCAGC TCGTCGCTAT CGTGC GCGAG TATACTCAGA GATTCCTCCG AAGTCGCATG	5040
CGTGC GTTG GTTTGAATAA CGAGTTCGTG GTAAAATCTT TCGCCGATTT GCTACCCGCA	5100
TTAATGAAGC GGAAGGTTTC AGGTTTCGTT TTAGCTAGTG TTTATCGCCC ACTTAGAGGT	5160
TTCTCATATA TGTGTGTTTC AGCGGAGCGA CGTGAAAAGT TTTTGTCTCT CGTGTGTTTA	5220
ATCGGGTTAA GTCTCCCTTT CTTCGTGCGC ATCGTAGGAG CGAAAGCGTG CGAAGAACTC	5280
GTGTCCTCAG CGCGTCGCTT TTATGAGCGT ATTAAAAATT TTCTAAGGCA GAAGTATGTC	5340
TCTCTTTCTA ATTTCTTTTG TCACTTGTTT AGCTCTGACG TTGATGACAG TTCCGCATCT	5400
GCAGGGTTGA AAGGTGGTGC GTCGCGAATG ACGCTCTTCC ACCTTCTGGT TCGCCTTGCT	5460
AGTGCCCTCC TATCGTTAGG GTGGGAAGGG TTAAGCTAC TCTTATCGCA CCACAACCTG	5520
TTATTTTTGT GTTTGCAATT GGTGACGAT GTGAACGTCC TTATCAAAGT TCTTGGGGGT	5580
CTTCTTTTCT TTGTGCAACC AATCTTTTCC TTGTTGCGG CGATGCTTCT ACAACCGGAC	5640
AGGTTTGTGG AGTATTCGGA GAAACTTGTT ACAGCGTTTG AATTTTCTT AAAATGTTTCG	5700
CCTCGCGCGC CTGCACTACT CAAAGGGTTT TTTGAGTGGC TGGCGAACAG CACTGTGTCA	5760
AAAACCGTTC GAAGACTTCT TCGCTGTTTC GTGAAGATGC TCAAACCTCG AAAAGGGCGA	5820
GGGTGCGTG CGGATGGTAG GGGTCTCCAT CGGCAGAAAG CGTACCCGT CATACCTTCT	5880
AATCGGGTCG TGACCGACGG GGTGAAAGA CTTTCGGTAA AGATGCAAGG AGTTGAAGCG	5940

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TTGCGTACC	G AATTGAGAA	CTTAGAAGAT	TTAGATTCTG	CCGTGATCGA	AAAAC	CAAT	6000
AGACGCAGAA	ATCGTGACAC	TAATGACGAC	GAATTACGCG	GCCCTGCTCA	TGAGCAGATG		6060
CAAGAAAGTCA	CCACTTTCTG	TTCGAAAGCC	AACCTCTGCTG	GTTTGCCCT	GGAAAGGGCA		6120
GTGCTTGTGG	AAGACGCTAT	AAAGTCGGAG	AAACTTTCTA	AGACGGTTAA	TGAGATGGTG		6180
AGGAAAGGGA	GTACCAACAG	CGAAGAAAGT	GCCGTCGCTT	TGTCGGACGA	TGAAGCCGTG		6240
GAAGAAATCT	CTGTTGCTGA	CGAGCGAGAC	GATTGCGCTA	AGACAGTCAG	GATAAGCGAA		6300
TACCTAAATA	GGTTAAACTC	AAGCTTCGAA	TTCCCGAAGC	CTATTGTTGT	GGACGACAAC		6360
AAGGATACCG	GGGGTCTAAC	GAACGCCGTG	AGGGAGTTTT	ATTATATGCA	AGAAGCTTGCT		6420
CTTTTCGAAA	TCCACAGCAA	ACTGTGCACC	TACTACGATC	AACGCGCAT	AGTCAACTTC		6480
GATCGTTCCG	TAGCACCATG	CAGCGAAGAT	GCTCAGCTGT	ACGTACGGAA	GAACGGCTCA		6540
ACGATAGTGC	AGGGTAAAGA	GGTACGTTTG	CACATTAAAG	ATTTCACGA	TCACGATTTC		6600
CTGTTTGACG	GAATAATTTT	TATTAACAAG	CGCGGCGGAG	GCGGAAATGT	TTTATATCAC		6660
GACAACTTCG	CGTTCCTGGC	GAGTAAITTG	TTCTTAGCCG	GCTACCCCTT	TTCAGGAGC		6720
TTCGTCCTCA	CGAATTCGTC	GGTCGATATT	CTCCTCTACG	AAGCTCCACC	CGGAGGTGGT		6780
AAGACGACGA	CGCTGATTGA	CTCGTCTTTG	AAGGTCPTCA	AGAAAGGTGA	GTTTCCACC		6840
ATGATCTTAA	CCGCCACRAA	AAGTTCGCAG	GTTGAGATCC	TAAAGAAAGT	GGAGAAGGAA		6900
GTGTCTAACA	TTGAATGCCA	GAAACGTAAA	GACAAAAGAT	CTCCGAAAAA	GAGCATTTAC		6960
ACCATCGACG	CTTATTTAAT	GCATCACCCT	GGTGTGTATG	CAGACGTTCT	TTTCATCGAT		7020
GAGTGTTC	TGTTTCATGC	GGGTAGCGTA	CTAGCTTGCA	TTGAGTTTAC	GAGGTGTCTAT		7080
AAAGTAATGA	TCTTCGGGGA	TAGCCGCGAG	ATTCAC	TACATA	TTGAAAGGAA	CGAATTGGAG	7140
AAGTGT	TTTGTATCT	CGACAGGTT	CTGGAC	TTG	AGTGT	CGGGT	7200
ATTTG	TAAC	GTGTCATG	GCTT	GGTTAA	GCACAGT	GTA	7260
ATCGCACCG	TGAAGGGTGA	AAGCGAAGGT	AAGAGCAGCA	TGCGCATTA	CGAAATTAAT		7320
TCAGTCGACG	ATTTAGTCCC	CGACGTGGGT	TCCACGTTT	TGTGTATGCT	TCAGTCGGAG		7380
AAGTTG	GAAGCA	CTTTATTGCG	AAGGGTTTGA	CTAAACTTAA	CGTTCTAACG		7440
GTGCATGAGG	CGCAAGGTGA	GACGTATGCG	CGTGTGAACC	TTGTGCGACT	TAAGTTTCAG		7500
GAGGATGAAC	CCTTTAAATC	TATCAGGCAC	ATAACCGTCG	CTCTTCTCG	TCACCCGAC		7560
AGCTTAACTT	ATAACGCTTT	AGCTGCTCGT	CGAGGTGACG	CCACTTGC	GATGCCAG		7620
AAGGCTGCGG	AATTGGTGAA	CAAGTTTCG	GTTTTTCTTA	CATCTTTTGG	TGGTAGTGTT		7680
ATCAATCTCA	ACGTGAAGAA	GGACGTGGAA	GATAACAGTA	GGTGAAGGC	TTGTCGGCA		7740
CCATTGAGCG	TAATCAACGA	CTTTTGAAC	GAAGTTAATC	CCGGTACTGC	GGTGATTGAT		7800

The large polyprotein (papain-like proteases, methyltransferase, and helicase) has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

Thr 1	Leu	Arg 5	Glu	Asn 5	Pro	Ile	Ser	Val	Ser 10	Gly	Val	Asn	Leu	Gly 15	Arg
Ser	Ala	Ala 20	Ala	Gln	Val	Ile	Tyr	Phe	Gly 25	Ser	Phe	Thr	Gln 30	Pro	Phe
Ala	Leu	Tyr 35	Pro	Arg	Gln	Glu	Ser 40	Ala	Ile	Val	Lys	Thr 45	Gln	Leu	Pro
Pro	Val 50	Ser	Val	Val	Lys 55	Val	Glu	Cys	Val	Ala 60	Ala	Glu	Val	Ala	Pro
Asp 65	Arg	Gly	Val	Val 70	Asp	Lys	Lys	Pro	Thr	Ser 75	Val	Gly	Val	Pro	Pro 80
Gln	Arg	Gly	Val	Leu 85	Ser	Phe	Pro	Thr	Val 90	Val	Arg	Asn	Arg	Gly 95	Asp
Val	Ile	Ile	Thr 100	Gly	Val	Val	His 105	Glu	Ala	Leu	Lys	Lys 110	Ile	Lys	Asp
Gly	Leu 115	Leu	Arg	Phe	Arg	Val	Gly 120	Gly	Asp	Met	Arg	Phe 125	Ser	Arg	Phe
Phe	Ser 130	Ser	Asn	Tyr	Gly	Cys 135	Arg	Phe	Val	Ala 140	Ser	Val	Arg	Thr	Asn
Thr 145	Thr	Val	Trp	Leu	Asn 150	Cys	Thr	Lys	Ala	Ser 155	Gly	Glu	Lys	Phe	Ser 160
Leu	Ala	Ala	Ala 165	Cys	Thr	Ala	Asp	Tyr	Val 170	Ala	Met	Leu	Arg	Tyr	Val
Cys	Gly	Gly	Lys 180	Phe	Pro	Leu	Val	Leu 185	Met	Ser	Arg	Val	Ile 190	Tyr	Pro
Asp	Gly 195	Arg	Cys	Tyr	Leu	Ala	His 200	Met	Arg	Tyr	Leu	Cys 205	Ala	Phe	Tyr
Cys 210	Arg	Pro	Phe	Arg	Glu	Ser	Asp 215	Tyr	Ala	Leu 220	Gly	Met	Trp	Pro	Thr
Val 225	Ala	Arg	Leu	Arg	Ala 230	Cys	Val	Glu	Lys	Asn 235	Phe	Gly	Val	Glu	Ala 240
Cys	Gly	Ile	Ala 245	Leu	Arg	Gly	Tyr	Tyr	Thr 250	Ser	Arg	Asn	Val	Tyr 255	His
Cys	Asp 260	Tyr	Asp	Ser	Ala	Tyr	Val 265	Lys	Tyr	Phe	Arg	Asn 270	Leu	Ser	Gly

Arg Ile Gly Gly Gly Ser Phe Asp Pro Thr Ser Leu Thr Ser Val Ile
275 280 285

Thr Val Lys Ile Ser Gly Leu Pro Gly Gly Leu Pro Lys Asn Ile Ala
290 295 300

Phe Gly Ala Phe Leu Cys Asp Ile Arg Tyr Val Glu Pro Val Asp Ser
305 310 315 320

Gly Gly Ile Gln Ser Ser Val Lys Thr Lys Arg Glu Asp Ala His Arg
325 330 335

Thr Val Glu Glu Arg Ala Ala Gly Gly Ser Val Glu Gln Pro Arg Gln
340 345 350

Lys Arg Ile Asp Glu Lys Gly Cys Gly Arg Val Pro Ser Gly Gly Phe
355 360 365

Ser His Leu Leu Val Gly Asn Leu Asn Glu Val Arg Arg Lys Val Ala
370 375 380

Ala Gly Leu Leu Arg Phe Arg Val Gly Gly Asp Met Asp Phe His Arg
385 390 395 400

Ser Phe Ser Thr Gln Ala Gly His Arg Leu Leu Val Trp Arg Arg Ser
405 410 415

Ser Arg Ser Val Cys Leu Glu Leu Tyr Ser Pro Ser Lys Asn Phe Leu
420 425 430

Arg Tyr Asp Val Leu Pro Cys Ser Gly Asp Tyr Ala Ala Met Phe Ser
435 440 445

Phe Ala Ala Gly Gly Arg Phe Pro Leu Val Leu Met Thr Arg Ile Arg
450 455 460

Tyr Pro Asn Gly Phe Cys Tyr Leu Ala His Cys Arg Tyr Ala Cys Ala
465 470 475 480

Phe Leu Leu Arg Gly Phe Asp Pro Lys Arg Phe Asp Ile Gly Ala Phe
485 490 495

Pro Thr Ala Ala Lys Leu Arg Asn Arg Met Val Ser ~~Glu Leu Gly~~ Gln
500 505 510

Arg Ser Leu Gly Leu Asn Leu Tyr Gly Ala Tyr Thr Ser Arg Gly Val
515 520 525

Phe His Cys Asp Tyr Asp Ala Lys Phe Ile Lys Asp Leu Arg Leu Met
530 535 540

Ser Ala Val Ile Ala Gly Lys Asp Gly Val Glu Glu Val Val Pro Ser
545 550 555 560

Asp Ile Thr Pro Ala Met Lys Gln Lys Thr Ile Glu Ala Val Tyr Asp
565 570 575

Arg Leu Tyr Gly Gly Thr Asp Ser Leu Leu Lys Leu Ser Ile Glu Lys
580 585 590

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Asp	Leu	Ile	Asp	Phe	Lys	Asn	Asp	Val	Gln	Ser	Leu	Lys	Lys	Asp	Arg
595							600					605			
Pro	Ile	Val	Lys	Val	Pro	Phe	Tyr	Met	Ser	Glu	Ala	Thr	Gln	Asn	Ser
610						615					620				
Leu	Thr	Arg	Phe	Tyr	Pro	Gln	Phe	Glu	Leu	Lys	Phe	Ser	His	Ser	Ser
625					630					635					640
His	Ser	Asp	His	Pro	Ala	Ala	Ala	Ala	Ser	Arg	Leu	Leu	Glu	Asn	Glu
				645					650					655	
Thr	Leu	Val	Arg	Leu	Cys	Gly	Asn	Ser	Val	Ser	Asp	Ile	Gly	Gly	Cys
				660				665					670		
Pro	Leu	Phe	His	Leu	His	Ser	Lys	Thr	Gln	Arg	Arg	Val	His	Val	Cys
							680					685			
Arg	Pro	Val	Leu	Asp	Gly	Lys	Asp	Ala	Gln	Arg	Arg	Val	Val	Arg	Asp
	690					695					700				
Leu	Gln	Tyr	Ser	Asn	Val	Arg	Leu	Gly	Asp	Asp	Asp	Lys	Ile	Leu	Glu
705					710					715					720
Gly	Pro	Arg	Asn	Ile	Asp	Ile	Cys	His	Tyr	Pro	Leu	Gly	Ala	Cys	Asp
				725					730					735	
His	Glu	Ser	Ser	Ala	Met	Met	Met	Val	Gln	Val	Tyr	Asp	Ala	Ser	Leu
				740				745						750	
Tyr	Glu	Ile	Cys	Gly	Ala	Met	Ile	Lys	Lys	Lys	Ser	Arg	Ile	Thr	Tyr
	755						760					765			
Leu	Thr	Met	Val	Thr	Pro	Gly	Glu	Phe	Leu	Asp	Gly	Arg	Glu	Cys	Val
	770					775					780				
Tyr	Met	Glu	Ser	Leu	Asp	Cys	Glu	Ile	Glu	Val	Asp	Val	His	Ala	Asp
785					790					795					800
Val	Val	Met	Tyr	Lys	Phe	Gly	Ser	Ser	Cys	Tyr	Ser	His	Lys	Leu	Ser
				805					810					815	
Ile	Ile	Lys	Asp	Ile	Met	Thr	Thr	Phe	Tyr	Leu	Thr	Leu	Gly	Gly	Phe
			820					825					830		
Leu	Phe	Ser	Val	Glu	Met	Tyr	Glu	Val	Arg	Met	Gly	Val	Asn	Tyr	Phe
							840					845			
Lys	Ile	Thr	Lys	Ser	Glu	Val	Ser	Pro	Ser	Ile	Ser	Cys	Thr	Lys	Leu
	850					855					860				
Leu	Arg	Tyr	Arg	Arg	Ala	Asn	Ser	Asp	Val	Val	Lys	Val	Lys	Leu	Pro
865					870					875					880
Arg	Phe	Asp	Lys	Lys	Arg	Arg	Met	Cys	Leu	Pro	Gly	Tyr	Asp	Thr	Ile
				885					890					895	
Tyr	Leu	Asp	Ser	Lys	Phe	Val	Ser	Arg	Val	Phe	Asp	Tyr	Val	Val	Cys
			900					905					910		

Asn	Cys	Ser	Ala	Val	Asn	Ser	Lys	Thr	Phe	Glu	Trp	Val	Trp	Ser	Phe	
	915						920					925				
Ile	Lys	Ser	Ser	Lys	Ser	Arg	Val	Ile	Ile	Ser	Gly	Lys	Ile	Ile	His	
	930					935					940					
Lys	Asp	Val	Asn	Leu	Asp	Leu	Lys	Tyr	Val	Glu	Ser	Phe	Ala	Ala	Val	
	945				950					955					960	
Met	Leu	Ala	Ser	Gly	Val	Arg	Ser	Arg	Leu	Ala	Ser	Glu	Tyr	Leu	Ala	
				965					970					975		
Lys	Asn	Leu	Ser	His	Phe	Ser	Gly	Asp	Cys	Ser	Phe	Ile	Glu	Ala	Thr	
			980					985					990			
Ser	Phe	Val	Leu	Arg	Glu	Lys	Ile	Arg	Asn	Met	Thr	Leu	Asn	Phe	Asn	
		995					1000						1005			
Glu	Arg	Leu	Leu	Gln	Leu	Val	Lys	Arg	Val	Ala	Phe	Ala	Thr	Leu	Asp	
	1010					1015						1020				
Val	Ser	Phe	Leu	Asp	Leu	Asp	Ser	Thr	Leu	Glu	Ser	Ile	Thr	Asp	Phe	
	1025				1030					1035					1040	
Ala	Glu	Cys	Lys	Val	Ala	Ile	Glu	Leu	Asp	Glu	Leu	Gly	Cys	Leu	Arg	
				1045					1050					1055		
Ala	Glu	Ala	Glu	Asn	Glu	Lys	Ile	Arg	Asn	Leu	Ala	Gly	Asp	Ser	Ile	
			1060					1065					1070			
Ala	Ala	Lys	Leu	Ala	Ser	Glu	Ile	Val	Val	Asp	Ile	Asp	Ser	Lys	Pro	
		1075					1080					1085				
Ser	Pro	Lys	Gln	Val	Gly	Asn	Ser	Ser	Ser	Glu	Asn	Ala	Asp	Lys	Arg	
	1090					1095					1100					
Glu	Val	Gln	Arg	Pro	Gly	Leu	Arg	Gly	Gly	Ser	Arg	Asn	Gly	Val	Val	
	1105				1110					1115					1120	
Gly	Glu	Phe	Leu	His	Phe	Val	Val	Asp	Ser	Ala	Leu	Arg	Leu	Phe	Lys	
				1125					1130					1135		
Tyr	Ala	Thr	Asp	Gln	Gln	Arg	Ile	Lys	Ser	Tyr	Val	Arg	Phe	Leu	Asp	
			1140					1145						1150		
Ser	Ala	Val	Ser	Phe	Leu	Asp	Tyr	Asn	Tyr	Asp	Asn	Leu	Ser	Phe	Ile	
		1155					1160					1165				
Leu	Arg	Val	Leu	Ser	Glu	Gly	Tyr	Ser	Cys	Met	Phe	Ala	Phe	Leu	Ala	
	1170					1175					1180					
Asn	Arg	Gly	Asp	Leu	Ser	Ser	Arg	Val	Arg	Ser	Ala	Val	Cys	Ala	Val	
	1185				1190					1195					1200	
Lys	Glu	Val	Ala	Thr	Ser	Cys	Ala	Asn	Ala	Ser	Val	Ser	Lys	Ala	Lys	
				1205					1210					1215		
Val	Met	Ile	Thr	Phe	Ala	Ala	Ala	Val	Cys	Ala	Met	Met	Phe	Asn	Ser	
			1220					1225					1230			

Cys Gly Phe Ser Gly Asp Gly Arg Glu Tyr Lys Ser Tyr Ile His Arg
 1235 1240 1245
 Tyr Thr Gln Val Leu Phe Asp Thr Ile Phe Phe Glu Asp Ser Ser Tyr
 1250 1255 1260
 Leu Pro Ile Glu Val Leu Ser Ser Ala Ile Cys Gly Ala Ile Val Thr
 1265 1270 1275 1280
 Leu Phe Ser Ser Gly Ser Ser Ile Ser Leu Asn Ala Phe Leu Leu Gln
 1285 1290 1295
 Ile Thr Lys Gly Phe Ser Leu Glu Val Val Arg Asn Val Val Arg
 1300 1305 1310
 Val Thr His Gly Leu Ser Thr Thr Ala Thr Asp Gly Val Ile Arg Gly
 1315 1320 1325
 Val Phe Ser Gln Ile Val Ser His Leu Leu Val Gly Asn Thr Gly Asn
 1330 1335 1340
 Val Ala Tyr Gln Ser Ala Phe Ile Ala Gly Val Val Pro Leu Leu Val
 1345 1350 1355
 Lys Lys Cys Val Ser Leu Ile Phe Ile Leu Arg Glu Asp Thr Tyr Ser
 1365 1370 1375
 Gly Phe Ile Lys His Gly Ile Ser Glu Phe Ser Phe Leu Ser Ser Ile
 1380 1385 1390
 Leu Lys Phe Leu Lys Gly Lys Leu Val Asp Glu Leu Lys Ser Ile Ile
 1395 1400 1405
 Gln Gly Val Phe Asp Ser Asn Lys His Val Phe Lys Glu Ala Thr Gln
 1410 1415 1420
 Glu Ala Ile Arg Thr Thr Val Met Gln Val Pro Val Ala Val Val Asp
 1425 1430 1435 1440
 Ala Leu Lys Ser Ala Ala Gly Lys Ile Tyr Asn Asn Phe Thr Ser Arg
 1445 1450 1455
 Arg Thr Phe Gly Lys Asp Glu Gly Ser Ser Ser Asp Gly Ala Cys Glu
 1460 1465 1470
 Glu Tyr Phe Ser Cys Asp Glu Gly Glu Gly Pro Gly Leu Lys Gly Gly
 1475 1480 1485
 Ser Ser Tyr Gly Phe Ser Ile Leu Ala Phe Phe Ser Arg Ile Met Trp
 1490 1495 1500
 Gly Ala Arg Arg Leu Ile Val Lys Val Lys His Glu Cys Phe Gly Lys
 1505 1510 1515 1520
 Leu Phe Glu Phe Leu Ser Leu Lys Leu His Glu Phe Arg Thr Arg Val
 1525 1530 1535
 Phe Gly Lys Asn Arg Thr Asp Val Gly Val Tyr Asp Phe Leu Pro Thr
 1540 1545 1550

- -

Gly Ile Val Glu Thr Leu Ser Ser Ile Glu Glu Cys Asp Gln Ile Glu
1555 1560 1565

Glu Leu Leu Gly Asp Asp Leu Lys Gly Asp Lys Asp Ala Ser Leu Thr
1570 1575 1580

Asp Met Asn Tyr Phe Glu Phe Ser Glu Asp Phe Leu Ala Ser Ile Glu
1585 1590 1595 1600

Glu Pro Pro Phe Ala Gly Leu Arg Gly Gly Ser Lys Asn Ile Ala Ile
1605 1610 1615

Leu Ala Ile Leu Glu Tyr Ala His Asn Leu Phe Arg Ile Val Ala Ser
1620 1625 1630

Lys Cys Ser Lys Arg Pro Leu Phe Leu Ala Phe Ala Glu Leu Ser Ser
1635 1640 1645

Ala Leu Ile Glu Lys Phe Lys Glu Val Phe Pro Arg Lys Ser Gln Leu
1650 1655 1660

Val Ala Ile Val Arg Glu Tyr Thr Gln Arg Phe Leu Arg Ser Arg Met
1665 1670 1675 1680

Arg Ala Leu Gly Leu Asn Asn Glu Phe Val Val Lys Ser Phe Ala Asp
1685 1690 1695

Leu Leu Pro Ala Leu Met Lys Arg Lys Val Ser Gly Ser Phe Leu Ala
1700 1705 1710

Ser Val Tyr Arg Pro Leu Arg Gly Phe Ser Tyr Met Cys Val Ser Ala
1715 1720 1725

Glu Arg Arg Glu Lys Phe Phe Ala Leu Val Cys Leu Ile Gly Leu Ser
1730 1735 1740

Leu Pro Phe Phe Val Arg Ile Val Gly Ala Lys Ala Cys Glu Glu Leu
1745 1750 1755 1760

Val Ser Ser Ala Arg Arg Phe Tyr Glu Arg Ile Lys Ile Phe Leu Arg
1765 1770 1775

Gln Lys Tyr Val Ser Leu Ser Asn Phe Phe Cys His Leu Phe Ser Ser
1780 1785 1790

Asp Val Asp Asp Ser Ser Ala Ser Ala Gly Leu Lys Gly Gly Ala Ser
1795 1800 1805

Arg Met Thr Leu Phe His Leu Leu Val Arg Leu Ala Ser Ala Leu Leu
1810 1815 1820

Ser Leu Gly Trp Glu Gly Leu Lys Leu Leu Ser His His Asn Leu
1825 1830 1835 1840

Leu Phe Leu Cys Phe Ala Leu Val Asp Asp Val Asn Val Leu Ile Lys
1845 1850 1855

Val Leu Gly Gly Leu Ser Phe Phe Val Gln Pro Ile Phe Ser Leu Phe
1860 1865 1870

001100 000000 000000

Ala	Ala	Met	Leu	Leu	Gln	Pro	Asp	Arg	Phe	Val	Glu	Tyr	Ser	Glu	Lys
1875							1880					1885			
Leu	Val	Thr	Ala	Phe	Glu	Phe	Phe	Leu	Lys	Cys	Ser	Pro	Arg	Ala	Pro
1890						1895					1900				
Ala	Leu	Leu	Lys	Gly	Phe	Phe	Glu	Cys	Val	Ala	Asn	Ser	Thr	Val	Ser
1905					1910					1915					1920
Lys	Thr	Val	Arg	Arg	Leu	Leu	Arg	Cys	Phe	Val	Lys	Met	Leu	Lys	Leu
				1925					1930					1935	
Arg	Lys	Gly	Arg	Gly	Leu	Arg	Ala	Asp	Gly	Arg	Gly	Leu	His	Arg	Gln
			1940					1945					1950		
Lys	Ala	Val	Pro	Val	Ile	Pro	Ser	Asn	Arg	Val	Val	Thr	Asp	Gly	Val
			1955				1960						1965		
Glu	Arg	Leu	Ser	Val	Lys	Met	Gln	Gly	Val	Glu	Ala	Leu	Arg	Thr	Glu
1970						1975					1980				
Leu	Arg	Ile	Leu	Glu	Asp	Leu	Asp	Ser	Ala	Val	Ile	Glu	Lys	Leu	Asn
1985					1990					1995					2000
Arg	Arg	Arg	Asn	Arg	Asp	Thr	Asn	Asp	Asp	Glu	Phe	Thr	Arg	Pro	Ala
				2005					2010					2015	
His	Glu	Gln	Met	Gln	Glu	Val	Thr	Thr	Phe	Cys	Ser	Lys	Ala	Asn	Ser
				2020				2025					2030		
Ala	Gly	Leu	Ala	Leu	Glu	Arg	Ala	Val	Leu	Val	Glu	Asp	Ala	Ile	Lys
			2035				2040					2045			
Ser	Glu	Lys	Leu	Ser	Lys	Thr	Val	Asn	Glu	Met	Val	Arg	Lys	Gly	Ser
						2055					2060				
Thr	Thr	Ser	Glu	Glu	Val	Ala	Val	Ala	Leu	Ser	Asp	Asp	Glu	Ala	Val
2065					2070						2075				2080
Glu	Glu	Ile	Ser	Val	Ala	Asp	Glu	Arg	Asp	Asp	Ser	Pro	Lys	Thr	Val
				2085					2090					2095	
Arg	Ile	Ser	Glu	Tyr	Leu	Asn	Arg	Leu	Asn	Ser	Ser	Phe	Glu	Phe	Pro
			2100					2105					2110		
Lys	Pro	Ile	Val	Val	Asp	Asp	Asn	Lys	Asp	Thr	Gly	Gly	Leu	Thr	Asn
			2115					2120				2125			
Ala	Val	Arg	Glu	Phe	Tyr	Tyr	Met	Gln	Glu	Leu	Ala	Leu	Phe	Glu	Ile
						2135					2140				
His	Ser	Lys	Leu	Cys	Thr	Tyr	Tyr	Asp	Gln	Leu	Arg	Ile	Val	Asn	Phe
2145					2150					2155					2160
Asp	Arg	Ser	Val	Ala	Pro	Cys	Ser	Glu	Asp	Ala	Gln	Leu	Tyr	Val	Arg
				2165					2170					2175	
Lys	Asn	Gly	Ser	Thr	Ile	Val	Gln	Gly	Lys	Glu	Val	Arg	Leu	His	Ile
			2180					2185					2190		

Lys Asp Phe His Asp His Asp Phe Leu Phe Asp Gly Lys Ile Ser Ile
 2195 2200 2205
 Asn Lys Arg Arg Arg Gly Gly Asn Val Leu Tyr His Asp Asn Leu Ala
 2210 2215 2220
 Phe Leu Ala Ser Asn Leu Phe Leu Ala Gly Tyr Pro Phe Ser Arg Ser
 2225 2230 2235 2240
 Phe Val Phe Thr Asn Ser Ser Val Asp Ile Leu Leu Tyr Glu Ala Pro
 2245 2250 2255
 Pro Gly Gly Gly Lys Thr Thr Thr Leu Ile Asp Ser Phe Leu Lys Val
 2260 2265 2270
 Phe Lys Lys Gly Glu Val Ser Thr Met Ile Leu Thr Ala Asn Lys Ser
 2275 2280 2285
 Ser Gln Val Glu Ile Leu Lys Lys Val Glu Lys Glu Val Ser Asn Ile
 2290 2295 2300
 Glu Cys Gln Lys Arg Lys Asp Lys Arg Ser Pro Lys Lys Ser Ile Tyr
 2305 2310 2315 2320
 Thr Ile Asp Ala Tyr Leu Met His His Arg Gly Cys Asp Ala Asp Val
 2325 2330 2335
 Leu Phe Ile Asp Glu Cys Phe Met Val His Ala Gly Ser Val Leu Ala
 2340 2345 2350
 Cys Ile Glu Phe Thr Arg Cys His Lys Val Met Ile Phe Gly Asp Ser
 2355 2360 2365
 Arg Gln Ile His Tyr Ile Glu Arg Asn Glu Leu Asp Lys Cys Leu Tyr
 2370 2375 2380
 Gly Asp Leu Asp Arg Phe Val Asp Leu Gln Cys Arg Val Tyr Gly Asn
 2385 2390 2395 2400
 Ile Ser Tyr Arg Cys Pro Trp Asp Val Cys Ala Trp Leu Ser Thr Val
 2405 2410 2415
 Tyr Gly Asn Leu Ile Ala Thr Val Lys Gly Glu Ser Glu Gly Lys Ser
 2420 2425 2430
 Ser Met Arg Ile Asn Glu Ile Asn Ser Val Asp Asp Leu Val Pro Asp
 2435 2440 2445
 Val Gly Ser Thr Phe Leu Cys Met Leu Gln Ser Glu Lys Leu Glu Ile
 2450 2455 2460
 Ser Lys His Phe Ile Arg Lys Gly Leu Thr Lys Leu Asn Val Leu Thr
 2465 2470 2475 2480
 Val His Glu Ala Gln Gly Glu Thr Tyr Ala Arg Val Asn Leu Val Arg
 2485 2490 2495
 Leu Lys Phe Gln Glu Asp Glu Pro Phe Lys Ser Ile Arg His Ile Thr
 2500 2505 2510

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Ile Val Val Arg Asp
2620
2636 2637 2639 2630
Lys Gln Arg Val

5

AGCGTAGTTC	GGTCGCAGGC	GATTCCGCGT	AGAAAACCTT	CTCTACAAGA	AAATTGTGAT	60
TCGTTTGAAG	CGCGGAATTA	TAACTTCTCG	ACTTGCGACC	GTAACACATC	TGCTTCAATG	120
TTCGGAGAGG	CTATGGCGAT	GAACTGTCTT	CGTCGTGTCT	TCGACCTAGA	TGCCTTTTCG	180
TCCCTGCGTG	ATGATGTGAT	TAGTATCACA	CGTTCAGGCA	TCGAACAATG	GCTGGAGAAA	240
CGTACTCCTA	GTCAGATTAA	AGCATTAAATG	AAGGATGTTG	AATCGCCTTT	GGAATTGAC	300
GATGAAATTT	TGCTGTTTAA	GTTGATGGTG	AAGCGTGACG	CTAAGGTGAA	GTTAGACTCT	360
TCTGTTTAA	CTAAACACAG	CGCCGCTCAA	AATATCATGT	TTCATCGCAA	GAGCATTAAT	420
GCTATCTTCT	CTCCTATCTT	TAATGAGGTG	AAAAACCGAA	TAATGTGCTG	TCTTAAGCCT	480
AACATAAAGT	TTTTTACGGA	GATGACTAAC	AGGGATTTTG	CTTCTGTTGT	CAGCAACATG	540
CTTGGTGACG	ACGATGTGTA	CCATATAGGT	GAAGTTGATT	TCTCAAAGTA	CGACAAGTCT	600
CAAGATGCTT	TCGTGAAGGC	TTTTGAAGAA	GTAATGTATA	AGGAACTCGG	TGTTGATGAA	660
GAGTTGCTGG	CTATCTGGAT	GTGCGGCGAG	CGGTTATCGA	TAGCTAACAC	TCTCGATGGT	720
CAGTTGTCCT	TCACGATCGA	GAATCAAAGG	AAGTCGGGAG	CTTCGAACAC	TTGGATTGGT	780

AACTCTCTCG TCACCTTTGGG TATTTTAAGT CTTTACTACG ACGTTAGAAA TTTCGAGGCG	840
TTGTACATCT CGGGCGATGA TTCTTTAATT TTTTCTCGCA GCGAGATTTT GAATTATGCC	900
GACGACATAT GCACTGACAT GGGTTTTGAG ACAAATTTA TGTCCCCAAG TGTCCCGTAC	960
TTTTGTCTTA AATTTGTTGT TATGTGTGGT CATAAGACGT TTTTGTGTCC CGACCCGTAC	1020
AAGCTTTTTG TCAAGTTGGG AGCAGTCAAA GAGGATGTTT CAATGGATTT CCTTTTCGAG	1080
ACTTTTACCT CCTTTAAGA CTTAACCTCC GATTTTAACG ACGAGCGCTT AATTCAAAG	1140
CTCGCTGAAC TTGTGGCTTT AAAATATGAG GTTCAAACCG GCAACACCAC CTGGCGTTA	1200
AGTGTGATAC ATTGTTTGGC TTCGAATTC CTCTCGTTTA GCAAGTTATA TCCTCGCGTG	1260
AAGGGATGGC AGGTTTTTTA CACGTCGGTT AAGAAAGCGC TTCTCAAGAG TGGGTGTCTCT	1320
CTCTTCGACA GTTTCATGAC CCCTTTTGGT CAGGCTGTCA TGGTTTGGGA TGATGAGTAG	1380

The RNA-dependent RNA polymerase has an amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

Ser Val Val Arg	Ser Gln Ala	Ile Pro	Arg Arg Lys	Pro Ser Leu Gln
1	5		10	15
Glu Asn Leu Tyr	Ser Phe Glu	Ala Arg	Asn Tyr Asn Phe	Ser Thr Cys
	20		25	30
Asp Arg Asn Thr	Ser Ala Ser	Met Phe Gly	Glu Ala Met	Ala Met Asn
	35		40	45
Cys Leu Arg Arg	Cys Phe Asp	Leu Asp Ala	Phe Ser Ser	Leu Arg Asp
	50		55	60
Asp Val Ile Ser	Ile Thr Arg	Ser Gly Ile	Glu Gln Trp	Leu Glu Lys
	65		70	75
Arg Thr Pro Ser	Gln Ile Lys	Ala Leu Met	Lys Asp Val	Glu Ser Pro
	85		90	95
Leu Glu Ile Asp	Asp Glu Ile	Cys Arg Phe	Lys Leu Met	Val Lys Arg
	100		105	110
Asp Ala Lys Val	Lys Leu Asp	Ser Ser Cys	Leu Thr Lys	His Ser Ala
	115		120	125
Ala Gln Asn Ile	Met Phe His	Arg Lys Ser	Ile Asn Ala	Ile Phe Ser
	130		135	140
Pro Ile Phe Asn	Glu Val Lys	Asn Arg Ile	Met Cys Cys	Leu Lys Pro
	145		150	155
Asn Ile Lys Phe	Phe Thr Glu	Met Thr Asn	Arg Asp Phe	Ala Ser Val
	165		170	175
Val Ser Asn Met	Leu Gly Asp	Asp Val Tyr	His Ile Gly	Glu Val
	180		185	190

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Asp	Phe	Ser	Lys	Tyr	Asp	Lys	Ser	Gln	Asp	Ala	Phe	Val	Lys	Ala	Phe
		195					200					205			
Glu	Glu	Val	Met	Tyr	Lys	Glu	Leu	Gly	Val	Asp	Glu	Glu	Leu	Leu	Ala
	210					215					220				
Ile	Trp	Met	Cys	Gly	Glu	Arg	Leu	Ser	Ile	Ala	Asn	Thr	Leu	Asp	Gly
	225				230					235					240
Gln	Leu	Ser	Phe	Thr	Ile	Glu	Asn	Gln	Arg	Lys	Ser	Gly	Ala	Ser	Asn
				245					250					255	
Thr	Trp	Ile	Gly	Asn	Ser	Leu	Val	Thr	Leu	Gly	Ile	Leu	Ser	Leu	Tyr
			260					265					270		
Tyr	Asp	Val	Arg	Asn	Phe	Glu	Ala	Leu	Tyr	Ile	Ser	Gly	Asp	Asp	Ser
		275					280					285			
Leu	Ile	Phe	Ser	Arg	Ser	Glu	Ile	Ser	Asn	Tyr	Ala	Asp	Asp	Ile	Cys
	290					295					300				
Thr	Asp	Met	Gly	Phe	Glu	Thr	Lys	Phe	Met	Ser	Pro	Ser	Val	Pro	Tyr
	305				310					315					320
Phe	Cys	Ser	Lys	Phe	Val	Val	Met	Cys	Gly	His	Lys	Thr	Phe	Phe	Val
				325					330					335	
Pro	Asp	Pro	Tyr	Lys	Leu	Phe	Val	Lys	Leu	Gly	Ala	Val	Lys	Glu	Asp
			340					345					350		
Val	Ser	Met	Asp	Phe	Leu	Phe	Glu	Thr	Phe	Thr	Ser	Phe	Lys	Asp	Leu
		355					360					365			
Thr	Ser	Asp	Phe	Asn	Asp	Glu	Arg	Leu	Ile	Gln	Lys	Leu	Ala	Glu	Leu
	370				375						380				
Val	Ala	Leu	Lys	Tyr	Glu	Val	Gln	Thr	Gly	Asn	Thr	Thr	Leu	Ala	Leu
	385				390					395					400
Ser	Val	Ile	His	Cys	Leu	Arg	Ser	Asn	Phe	Leu	Ser	Phe	Ser	Lys	Leu
			405						410					415	
Tyr	Pro	Arg	Val	Lys	Gly	Trp	Gln	Val	Phe	Tyr	Thr	Ser	Val	Lys	Lys
			420				425						430		
Ala	Leu	Leu	Lys	Ser	Gly	Cys	Ser	Leu	Phe	Asp	Ser	Phe	Met	Thr	Pro
	435						440					445			
Phe	Gly	Gln	Ala	Val	Met	Val	Trp	Asp	Asp	Glu					
	450					455									

and a molecular weight from about 50 to about 54 kDa, preferably about 52 kDa.

Another such DNA molecule (GLRAV-2 ORF2) includes nucleotides 9365-9535 of SEQ. ID. No. 1 and codes for a small, grapevine leafroll virus hydrophobic protein or polypeptide. This DNA molecule comprises the nucleotide sequence

5 corresponding to SEQ. ID. No. 6 as follows:

ATGAATCAGG TTTTGCAGTT TGAATGTTTG TTCTGCTGA ATCTCGCGGT TTTTGTCTGT 60
 ACTTTCATTT TCATTCTTCT GGTCTTCCGC GTGATTAAAG CTTTTCGCCA GAAGGGTCAC 120
 GAAGCACCTG TTCCCGTTGT TCGTGGCGGG GGTTTTTCAA CCGTAGTGTA G 171

The small hydrophobic protein or polypeptide has an amino acid sequence corresponding to SEQ. ID. No. 7 as follows:

Met	Asn	Gln	Val	Leu	Gln	Phe	Glu	Cys	Leu	Phe	Leu	Leu	Asn	Leu	Ala
1				5					10					15	
Val	Phe	Ala	Val	Thr	Phe	Ile	Phe	Ile	Leu	Leu	Val	Phe	Arg	Val	Ile
			20					25					30		
Lys	Ser	Phe	Arg	Gln	Lys	Gly	His	Glu	Ala	Pro	Val	Pro	Val	Val	Arg
			35				40					45			
Gly	Gly	Gly	Phe	Ser	Thr	Val	Val								
		50				55									

and a molecular weight from about 5 to about 7 kDa, preferably about 6 kDa.

- Another such DNA molecule (GLRaV-2 ORF3) includes nucleotides
 5 9551-11350 of SEQ. ID. No. 1 and encodes for a grapevine leafroll virus heat shock 70 protein. This DNA molecule comprises the nucleotide sequence corresponding to SEQ. ID. No. 8 as follows:

ATGGTAGTTT TCGGTTTGA CTTTGGCACC ACATTCTCTA CGGTGTGTGT GTACAAGGAT 60
 GGACGAGTTT TTTTCATTCA GCAGAATAAT TCGGCGTACA TCCCCACTTA CCTCTATCTC 120
 TTCTCCGATT CTAACCATAT GACTTTTGGT TACGAGSCCG AATCACTGAT GAGTAATCTG 180
 AAGTTAAAG GTTCGTTTTA TAGAGATTTA AATCGTTGGG TGGGTTGCGA TTCGAGTAAC 240
 CTCGACGCGT ACCTTGACCG TTTAAACCT CATTACTCGG TCCGCTTGCT TAAGATCGGC 300
 TCTGGCTTGA ACGAAACTGT TTCAATTGGA AACTTCGGGG GCACTGTATA GTCTGAGGCT 360
 CATCTGCCAG GGTGATAGC TCTCTTATT AAGGCTGTCA TTAGTTGCGC GGAGGGCGCG 420
 TTTGCGTGCA CTTGCACCGG GGTATTATTG TCAGTACCTG CCAATTATGA TAGCGTTCAA 480
 AGGAATTTC A CTGATCAGTG TGTTCACCTC AGCGGTTATC AGTGCGTATA TATGATCAAT 540
 GAACCTTCAG CGGCTCGCCT ATCTGCGTGT AATTCGATTG GAAAGAAGTC CGCAAATTTG 600
 GCTGTTTACG ATTTGCGTGG TGGGACCTTC GACGTGTCTA TCATTTCATA CCGCAACAAT 660
 ACTTTTGTGG TGCGAGCTTC TGGAGGCGAT CTAATCTCG GTGGAAGGGA TGTTGATCGT 720
 GCGTTTCTCA CGCACCTCTT CTCTTTAACA TCGCTGGAAC CTGACCTCAC TTTGGATATC 780

TCGAATCTGA AAGAATCTTT ATCAAAAACG GACGCAGAGA TAGTTTACAC TTTGAGAGGT 840
 GTCGATGGAA GAAAAGAAGA CGTTAGAGTA AACAAAAACA TTCTTACGTC GGTGATGCTC 900
 CCCTACGTGA ACAGAACGCT TAAGATATTA GAGTCAACCT TAAATCGTA TGCTAAGAGT 960
 ATGAATGAGA GTGCGCGAGT TAAGTCCGAT TTAGTGCTGA TAGGAGGATC TTCATATCTT 1020
 CCTGGCCTGG CAGACGTACT AACGAAGCAT CAGAGCGTTG ATCGTATCTT AAGAGTTTCG 1080
 GATCCTCGGG CTGCGGTGGC CGTCGGTTGC GCATTATATT CTTTCATGCTC CTCAGGATCT 1140
 GGGGGGTTGC TACTGATCGA CTGTGCAGCT CACACTGTCG CTATAGCGGA CAGAAGTTGT 1200
 CATCAAATCA TTTGCTCTCC AGCGGGGGCA CCGATCCCT TTTGAGGAG CATGCCCTTG 1260
 TACTTAGCCA GGGTCAACAA GAATCGCAG CGTGAAGTCG CCGTGTTTGA AGGGGAGTAC 1320
 GTTAAGTGCC CTAAGAACAG AAAGATCTGT GGAGCAAATA TAAGATTTTT TGATATAGGA 1380
 GTGACGGGTG ATTCGTACGC ACCCGTTACC TTCTATATGG ATTTCTCCAT TTCAAGCGTA 1440
 GGAGCCGTTT CATTCGTGGT GAGAGTCCCT GAGGGTAAGC AAGTGTCCT CACTGGAAGT 1500
 CCAGCGTATA ACTTTTCGTC TGTGGCTCTC GGATCAGCGA GTGTCCGAGA ATTGCATATT 1560
 AGTTTAAATA ATAAAGTTTT TCTCGGTTTG CTTCTACATA GAAAGCGGGA TCGACGAATA 1620
 CTTTCTACTA AGGATGAAGC GATTCGATAC GCCGATTCAA TTGATATCGC GGATGTGCTA 1680
 AAGGAATATA AAAGTTACGC GGCCAGTGCC TTACCACCAG ACGAGGATGT CGAATTACTC 1740
 CTGGGAAAGT CTGTTCAAAA AGTTTACGG GGAAGCAGAC TGGAAGAAAT ACCTCTCTAG 1800

The heat shock 70 protein is believed to function as a chaperone protein and has an amino acid sequence corresponding to SEQ. ID. No. 9 as follows:

Met Val Val Phe Gly Leu Asp Phe Gly Thr Thr Phe Ser Thr Val Cys
 1 5 10 15
 Val Tyr Lys Asp Gly Arg Val Phe Ser Phe Lys Gln Asn Asn Ser Ala
 20 25 30
 Tyr Ile Pro Thr Tyr Leu Tyr Leu Phe Ser Asp Ser Asn His Met Thr
 35 40 45
 Phe Gly Tyr Glu Ala Glu Ser Leu Met Ser Asn Leu Lys Val Lys Gly
 50 55 60
 Ser Phe Tyr Arg Asp Leu Lys Arg Trp Val Gly Cys Asp Ser Ser Asn
 65 70 75 80
 Leu Asp Ala Tyr Leu Asp Arg Leu Lys Pro His Tyr Ser Val Arg Leu
 85 90 95
 Val Lys Ile Gly Ser Gly Leu Asn Glu Thr Val Ser Ile Gly Asn Phe
 100 105 110

Gly	Gly	Thr	Val	Lys	Ser	Glu	Ala	His	Leu	Pro	Gly	Leu	Ile	Ala	Leu
115															
Phe	Ile	Lys	Ala	Val	Ile	Ser	Cys	Ala	Glu	Gly	Ala	Phe	Ala	Cys	Thr
130															
Cys	Thr	Gly	Val	Ile	Cys	Ser	Val	Pro	Ala	Asn	Tyr	Asp	Ser	Val	Gln
145															
Arg	Asn	Phe	Thr	Asp	Gln	Cys	Val	Ser	Leu	Ser	Gly	Tyr	Gln	Cys	Val
165															
Tyr	Met	Ile	Asn	Glu	Pro	Ser	Ala	Ala	Ala	Leu	Ser	Ala	Cys	Asn	Ser
180															
Ile	Gly	Lys	Lys	Ser	Ala	Asn	Leu	Ala	Val	Tyr	Asp	Phe	Gly	Gly	Gly
195															
Thr	Phe	Asp	Val	Ser	Ile	Ile	Ser	Tyr	Arg	Asn	Asn	Thr	Phe	Val	Val
210															
Arg	Ala	Ser	Gly	Gly	Asp	Leu	Asn	Leu	Gly	Gly	Arg	Asp	Val	Asp	Arg
225															
Ala	Phe	Leu	Thr	His	Leu	Phe	Ser	Leu	Thr	Ser	Leu	Glu	Pro	Asp	Leu
245															
Thr	Leu	Asp	Ile	Ser	Asn	Leu	Lys	Glu	Ser	Leu	Ser	Lys	Thr	Asp	Ala
260															
Glu	Ile	Val	Tyr	Thr	Leu	Arg	Gly	Val	Asp	Gly	Arg	Lys	Glu	Asp	Val
275															
Arg	Val	Asn	Lys	Asn	Ile	Leu	Thr	Ser	Val	Met	Leu	Pro	Tyr	Val	Asn
290															
Arg	Thr	Leu	Lys	Ile	Leu	Glu	Ser	Thr	Leu	Lys	Ser	Tyr	Ala	Lys	Ser
305															
Met	Asn	Glu	Ser	Ala	Arg	Val	Lys	Cys	Asp	Leu	Val	Leu	Ile	Gly	Gly
325															
Ser	Ser	Tyr	Leu	Pro	Gly	Leu	Ala	Asp	Val	Leu	Thr	Lys	His	Gln	Ser
340															
Val	Asp	Arg	Ile	Leu	Arg	Val	Ser	Asp	Pro	Arg	Ala	Ala	Val	Ala	Val
355															
Gly	Cys	Ala	Leu	Tyr	Ser	Ser	Cys	Leu	Ser	Gly	Ser	Gly	Gly	Leu	Leu
370															
Leu	Ile	Asp	Cys	Ala	Ala	His	Thr	Val	Ala	Ile	Ala	Asp	Arg	Ser	Cys
385															
His	Gln	Ile	Ile	Cys	Ala	Pro	Ala	Gly	Ala	Pro	Ile	Pro	Phe	Ser	Gly
405															
Ser	Met	Pro	Leu	Tyr	Leu	Ala	Arg	Val	Asn	Lys	Asn	Ser	Gln	Arg	Glu
420															

Val Ala Val Phe Glu Gly Glu Tyr Val Lys Cys Pro Lys Asn Arg Lys
435 440 445

Ile Cys Gly Ala Asn Ile Arg Phe Phe Asp Ile Gly Val Thr Gly Asp
450 455 460

Ser Tyr Ala Pro Val Thr Phe Tyr Met Asp Phe Ser Ile Ser Ser Val
465 470 475 480

Gly Ala Val Ser Phe Val Val Arg Gly Pro Glu Gly Lys Gln Val Ser
485 490 495

Leu Thr Gly Thr Pro Ala Tyr Asn Phe Ser Ser Val Ala Leu Gly Ser
500 505 510

Arg Ser Val Arg Glu Leu His Ile Ser Leu Asn Asn Lys Val Phe Lys
515 520 525

Gly Leu Leu Leu His Arg Lys Ala Asp Arg Arg Ile Leu Phe Thr Lys
530 535 540

Asp Glu Ala Ile Arg Tyr Ala Asp Ser Ile Asp Ile Ala Asp Val Leu
545 550 555 560

Lys Glu Tyr Lys Ser Tyr Ala Ala Ser Ala Leu Pro Pro Asp Glu Asp
565 570 575

Val Glu Leu Leu Leu Gly Lys Ser Val Gln Lys Val Leu Arg Gly Ser
580 585 590

Arg Leu Glu Glu Ile Pro Leu
595

and a molecular weight from about 63 to about 67 kDa, preferably about 65 kDa.

Another such DNA molecule (GLRaV-2 ORF4) includes nucleotides 11277-12932 of SEQ. ID. No. 1 and codes for a putative grapevine leafroll virus heat shock 90 protein. This DNA molecule comprises a nucleotide sequence corresponding to

5 SEQ. ID. No. 10 as follows:

ATGTCGAATT ACTCCTGGGA AAGTCTGTTC AAAAAGTTTT ACGGGGAAGC AGACTGGAAG	60
AAATACCTCT CTAGGAGCAT AGCAGCACAC TCAAGTGAAA TTAAACTCT ACCAGACATT	120
CGATTGTACG GCGGTAGGGT TGTAAGAAG TCCGAATTCG AATCAGCACT TCCTAATTCT	180
TTTGAACAGG AATTAGACT GTTCATACTG AGCGAACGGG AAGTGGGATG GAGCAAAATTA	240
TGCGGAATAA CGGTGGAAGA AGCAGCATAC GATCTTACGA ATCCCAAGGC TTATAAATTC	300
ACTGCCGAGA CATGTAGCCC GGATGTAAAA GGTGAAGGAC AAAAATACTC TATGGAAGAC	360
GTGATGAATT TCATGCGTTT ATCAAATCTG GATGTTAACG ACAAGATGCT GACGGAACAG	420
TGTTGGTCGC TGTCCAATTC ATGCGGTGAA TTGATCAACC CAGACGACAA AGGCGGATTC	480
GTGGCTCTCA CCTTTAAGGA CAGAGACACA GCTGATGACA CGGGTCCGCC CAACGTGGAA	540

TGTGCGGTGG GCGACTATCT AGTTTACGCT ATGTCCTGTG TTGAGCAGAG GACCCAAAAA 600
 TCGCAGTCTG GCAACATCTC TCTGTACGAA AAGTACTGTG AATACATCAG GACCTACTTA 660
 GGGAGTACAG ACCTGTTCTT CACAGCGCCG GACAGGATTC CGTTACTTAC GGGCATCCTA 720
 TACGATTTTT GTAAGGAATA CAACGTTTTT TACTCGTCAT ATAAGAGAAA CGTCGATAAT 780
 TTCAGATTCT TCTTGGCGAA TTATATGCCT TTGATATCTG ACGTCTTTGT CTTCAGTGG 840
 GTAAAACCCG CGCCGATGT TCGGCTGCTT TTTGAGTTAA GTGCAGCGGA ACTAACGCTG 900
 GAGGTTCCCA CACTGAGTTT GATAGATTCT CAAGTTGTGG TAGGTTCATAT CTTAAGATAC 960
 GTAGAATCCT ACACATCAGA TCCAGCCATC GACGCGTTAG AAGACAAACT GGAAGCGATA 1020
 CTGAAAAGTA GCAATCCCCG TCTATCGACA GCGCAACTAT GGGTTGGTTT CTTTGTGTAC 1080
 TATGGTGAGT TTCGTACGGC TCAAAGTAGA GTAGTGCAAA GACCAGGCGT ATACAAAACA 1140
 CCTGACTCAG TGGGTGGATT TGAATAAACC ATGAAAGATG TTGAGAAATT CTTGATAAAA 1200
 CTTAGAGAGG AATTGCCTAA TGTATCTTGG CGGCGTCAGT TTAACGGAGC TAGAGCGCAT 1260
 GAGGCTTTCA AAATATTTAA AACCGGAAT ATAAGTTTCA GACCTATATC GCGTTTAAAC 1320
 GTGCCATAGG AGTTCTGGTA TCTGAACATA GACTACTTCA GGCACGCGAA TAGGTCGGGG 1380
 TTAACCGAAG AAGAAATACT CATCCTAAAC AACATAAGCG TTGATGTTAG GAAGTTATGC 1440
 GCTGAGAGAG CGTGCAATAC CCTACCTAGC GCGAAGCGCT TTAGTAAAAA TCATAAGAGT 1500
 AATATACAAT CATCACGCCA AGAGCGGAGG ATTAAAGACC CATTGGTAGT CCTGAAAGAC 1560
 ACTTTATATG AGTTCCAACA CAAGCGTGCC GGTGGGGGT CTCGAAGCAC TCGAGACCTC 1620
 GGGAGTCGTG CTGACCACGC GAAAGGAAGC GGTGA 1656

The heat shock 90 protein has an amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

Met	Ser	Asn	Tyr	Ser	Trp	Glu	Ser	Leu	Phe	Lys	Lys	Phe	Tyr	Gly	Glu
1				5					10					15	
Ala	Asp	Trp	Lys	Lys	Tyr	Leu	Ser	Arg	Ser	Ile	Ala	Ala	His	Ser	Ser
			20					25					30		
Glu	Ile	Lys	Thr	Leu	Pro	Asp	Ile	Arg	Leu	Tyr	Gly	Gly	Arg	Val	Val
			35				40					45			
Lys	Lys	Ser	Glu	Phe	Glu	Ser	Ala	Leu	Pro	Asn	Ser	Ser	Phe	Glu	Gln
			50				55				60				
Leu	Gly	Leu	Phe	Ile	Leu	Ser	Glu	Arg	Glu	Val	Gly	Trp	Ser	Lys	Leu
					70					75					80

Cys Gly Ile Thr Val Glu Glu Ala Ala Tyr Asp Leu Thr Asn Pro Lys
 85 90 95
 Ala Tyr Lys Phe Thr Ala Glu Thr Cys Ser Pro Asp Val Lys Gly Glu
 100 105 110
 Gly Gln Lys Tyr Ser Met Glu Asp Val Met Asn Phe Met Arg Leu Ser
 115 120 125
 Asn Leu Asp Val Asn Asp Lys Met Leu Thr Glu Gln Cys Trp Ser Leu
 130 135 140
 Ser Asn Ser Cys Gly Glu Leu Ile Asn Pro Asp Asp Lys Gly Arg Phe
 145 150 155 160
 Val Ala Leu Thr Phe Lys Asp Arg Asp Thr Ala Asp Asp Thr Gly Ala
 165 170 175
 Ala Asn Val Glu Cys Arg Val Gly Asp Tyr Leu Val Tyr Ala Met Ser
 180 185 190
 Leu Phe Glu Gln Arg Thr Gln Lys Ser Gln Ser Gly Asn Ile Ser Leu
 195 200 205
 Tyr Glu Lys Tyr Cys Glu Tyr Ile Arg Thr Tyr Leu Gly Ser Thr Asp
 210 215 220
 Leu Phe Phe Thr Ala Pro Asp Arg Ile Pro Leu Leu Thr Gly Ile Leu
 225 230 235 240
 Tyr Asp Phe Cys Lys Glu Tyr Asn Val Phe Tyr Ser Ser Tyr Lys Arg
 245 250 255
 Asn Val Asp Asn Phe Arg Phe Phe Leu Ala Asn Tyr Met Pro Leu Ile
 260 265 270
 Ser Asp Val Phe Val Phe Gln Trp Val Lys Pro Ala Pro Asp Val Arg
 275 280 285
 Leu Leu Phe Glu Leu Ser Ala Ala Glu Leu Thr Leu Glu Val Pro Thr
 290 295 300
 Leu Ser Leu Ile Asp Ser Gln Val Val Val Gly His Ile Leu Arg Tyr
 305 310 315 320
 Val Glu Ser Tyr Thr Ser Asp Pro Ala Ile Asp Ala Leu Glu Asp Lys
 325 330 335
 Leu Glu Ala Ile Leu Lys Ser Ser Asn Pro Arg Leu Ser Thr Ala Gln
 340 345 350
 Leu Trp Val Gly Phe Phe Cys Tyr Tyr Gly Glu Phe Arg Thr Ala Gln
 355 360 365
 Ser Arg Val Val Gln Arg Pro Gly Val Tyr Lys Thr Pro Asp Ser Val
 370 375 380
 Gly Gly Phe Glu Ile Asn Met Lys Asp Val Glu Lys Phe Phe Asp Lys
 385 390 395 400

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Leu Gln Arg Glu Leu Pro Asn Val Ser Leu Arg Arg Gln Phe Asn Gly
405 410 415

Ala Arg Ala His Glu Ala Phe Lys Ile Phe Lys Asn Gly Asn Ile Ser
420 425 430

Phe Arg Pro Ile Ser Arg Leu Asn Val Pro Arg Glu Phe Trp Tyr Leu
435 440 445

Asn Ile Asp Tyr Phe Arg His Ala Asn Arg Ser Gly Leu Thr Glu Glu
450 455 460

Glu Ile Leu Ile Leu Asn Ile Ser Val Asp Val Arg Lys Leu Cys
465 470 475 480

Ala Glu Arg Ala Cys Asn Thr Leu Pro Ser Ala Lys Arg Phe Ser Lys
485 490 495

Asn His Lys Ser Asn Ile Gln Ser Ser Arg Gln Glu Arg Arg Ile Lys
500 505 510

Asp Pro Leu Val Val Leu Lys Asp Thr Leu Tyr Glu Phe Gln His Lys
515 520 525

Arg Ala Gly Trp Gly Ser Arg Ser Thr Arg Asp Leu Gly Ser Arg Ala
530 535 540

Asp His Ala Lys Gly Ser Gly
545 550

and a molecular weight from about 61 to about 65 kDa, preferably about 63 kDa.

Yet another DNA molecule of the present invention (GLRaV-2 ORF5) includes nucleotides 12844-13515 of SEQ. ID. No. 1 and codes for a diverged coat protein. This DNA molecule comprises a nucleotide sequence corresponding to SEQ. ID.

5 No. 12 as follows:

ATGAGTTCCA ACACAAGCGT GCCGGTTGGG GGTCCTCGAAG CACTCGAGAC CTCGGGAGTC 60

GTGCTGACCA CGCGAAAGGA AGCGGTTGAT AAGTTTTTTA ATGAACATAA AAACGAAAAAT 120

TACTCATCAG TTGACAGCAG CCGATTAAAG GATTTCGAAG TAAAGAAGT GTTAGAGAAA 180

AGTAAAGAAA GTTTCAAAG CGAACTGGCC TCCACTGACG AGCACTTCGT CTACCACATT 240

ATATTTTCT TAATCCGATG TGCTAAGATA TCGACAAGTG AAAAGGTGAA GTACGTTGGT 300

AGTCATACGT ACGTGGTCGA CGGAAAAACG TACACCGTTC TTGACGCTTG GGTATTCAAC 360

ATGATGAAAA GTCTCACGAA GAAGTACAAA CGAGTGAATG GTCTGCGTGC GTTCTGTTGC 420

GCGTGCGAAG ATCTATATCT AACCGTCGCA CCAATAATGT CAGAACGCTT TAAGACTAAA 480

GCCGTAGGGA TGAAAGGTTT GCCTGTTGGA AAGGAATACT TAGGCGCCGA CTTCTTTTCG 540

GGAAC TAGCA AACTGATGAG CGATCACGAC AGGGCGGTCT CCATCGTTGC AGCGAAAAAC 600

GCTGTCGATC GTAGCGCTTT CACGGGTGGG GAGAGAAAGA TAGTTAGTTT GTATGATCTA 660
GGGAGGTACT AA 672

The diverged coat protein has an amino acid sequence corresponding to SEQ. ID. No. 13 as follows:

Met	Ser	Ser	Asn	Thr	Ser	Val	Pro	Val	Gly	Gly	Leu	Glu	Ala	Leu	Glu	1	5	10	15
Thr	Ser	Gly	Val	Val	Leu	Thr	Thr	Arg	Lys	Glu	Ala	Val	Asp	Lys	Phe	20	25	30	35
Phe	Asn	Glu	Leu	Lys	Asn	Glu	Asn	Tyr	Ser	Ser	Val	Asp	Ser	Ser	Arg	35	40	45	
Leu	Ser	Asp	Ser	Glu	Val	Lys	Glu	Val	Leu	Glu	Lys	Ser	Lys	Glu	Ser	50	55	60	
Phe	Lys	Ser	Glu	Leu	Ala	Ser	Thr	Asp	Glu	His	Phe	Val	Tyr	His	Ile	65	70	75	80
Ile	Phe	Phe	Leu	Ile	Arg	Cys	Ala	Lys	Ile	Ser	Thr	Ser	Glu	Lys	Val	85	90	95	
Lys	Tyr	Val	Gly	Ser	His	Thr	Tyr	Val	Val	Asp	Gly	Lys	Thr	Tyr	Thr	100	105	110	
Val	Leu	Asp	Ala	Trp	Val	Phe	Asn	Met	Met	Lys	Ser	Leu	Thr	Lys	Lys	115	120	125	
Tyr	Lys	Arg	Val	Asn	Gly	Leu	Arg	Ala	Phe	Cys	Cys	Ala	Cys	Glu	Asp	130	135	140	
Leu	Tyr	Leu	Thr	Val	Ala	Pro	Ile	Met	Ser	Glu	Arg	Phe	Lys	Thr	Lys	145	150	155	160
Ala	Val	Gly	Met	Lys	Gly	Leu	Pro	Val	Gly	Lys	Glu	Tyr	Leu	Gly	Ala	165	170	175	
Asp	Phe	Leu	Ser	Gly	Thr	Ser	Lys	Leu	Met	Ser	Asp	His	Asp	Arg	Ala	180	185	190	
Val	Ser	Ile	Val	Ala	Ala	Lys	Asn	Ala	Val	Asp	Arg	Ser	Ala	Phe	Thr	195	200	205	
Gly	Gly	Glu	Arg	Lys	Ile	Val	Ser	Leu	Tyr	Asp	Leu	Gly	Arg	Tyr		210	215	220	

and a molecular weight from about 23 to about 27 kDa, preferably about 25 kDa.

- 5 Another such DNA molecule (GLRaV-2 ORF6) includes nucleotides 13584-14180 of SEQ. ID. No. 1 and codes for a grapevine leafroll virus coat protein. This DNA molecule comprises a nucleotide sequence corresponding to SEQ. ID. No. 14 as follows:

ATGGAGTTGA TGTCCGACAG CAACCTTAGC AACCTGGTGA TAACCGACGC CTCTAGTCTA	60
AATGGTGTGCG ACAAGAAGCT TTTATCTGCT GAAGTTGAAA AAATGTTGGT GCAGAAAGGG	120
GCTCCTAACG AGGGTATAGA AGTGGTGTTT GGTCTACTCC TTTACGCACT CGCGGCAAGA	180
ACCACGTCTC CTAAGGTTCA GCAGCGCAGAT TCAGACGTTA TATTTTCAAA TAGTTTCGGA	240
GAGAGGAATG TGGTAGTAAC AGAGGGTGAC CTTAAGAAGG TACTCGACGG GTGTGCGCCT	300
CTCACTAGGT TCACTAATAA ACTTAGAACG TTCGGTCGTA CTTTCACTGA GGCTTACGTT	360
GACTTTTGTA TCGCGTATAA GCACAAATTA CCCCAACTCA ACGCCGCGGC GGAATTGGGG	420
ATTCCAGCTG AAGATTCTGA CTTAGCTGCA GATTTTCTGG GTACTTGCCC GAAGCTCTCT	480
GAATTACAGC AAGTAGGAA GATGTTTCGC AGTATGTACG CTCTAAAAAC TGAAGGTGGA	540
GTGGTAAATA CACCACTGAG CAATCTGCGT CAGCTAGGTA GAAGGGAAGT TATGTAA	597

The coat protein has an amino acid sequence corresponding to SEQ. ID. No. 15 as follows:

Met Glu Leu Met Ser Asp Ser Asn Leu Ser Asn Leu Val Ile Thr Asp	
1 5 10 15	
Ala Ser Ser Leu Asn Gly Val Asp Lys Lys Leu Leu Ser Ala Glu Val	
20 25 30	
Glu Lys Met Leu Val Gln Lys Gly Ala Pro Asn Glu Gly Ile Glu Val	
35 40 45	
Val Phe Gly Leu Leu Leu Tyr Ala Leu Ala Ala Arg Thr Thr Ser Pro	
50 55 60	
Lys Val Gln Arg Ala Asp Ser Asp Val Ile Phe Ser Asn Ser Phe Gly	
65 70 75 80	
Glu Arg Asn Val Val Thr Glu Gly Asp Leu Lys Lys Val Leu Asp	
85 90 95	
Gly Cys Ala Pro Leu Thr Arg Phe Thr Asn Lys Leu Arg Thr Phe Gly	
100 105 110	
Arg Thr Phe Thr Glu Ala Tyr Val Asp Phe Cys Ile Ala Tyr Lys His	
115 120 125	
Lys Leu Pro Gln Leu Asn Ala Ala Glu Leu Gly Ile Pro Ala Glu	
130 135 140	
Asp Ser Tyr Leu Ala Ala Asp Phe Leu Gly Thr Cys Pro Lys Leu Ser	
145 150 155 160	
Glu Leu Gln Gln Ser Arg Lys Met Phe Ala Ser Met Tyr Ala Leu Lys	
165 170 175	

Thr Glu Gly Gly Val Val Asn Thr Pro Val Ser Asn Leu Arg Gln Leu
180 185 190

Gly Arg Arg Glu Val Met
195

and a molecular weight from about 20 to about 24 kDa, preferably about 22 kDa.

Another such DNA molecule (GLRaV-2 ORF7) includes nucleotides 14180-14665 of SEQ. ID. No. 1 and codes for a second undefined grapevine leafroll virus protein or polypeptide. This DNA molecule comprises a nucleotide sequence corresponding to SEQ. ID. No. 16 as follows:

ATGGAAGATT ACGAAGAAAA ATCCGAATCG CTCATACTGC TACGCACGAA TCTGAACACT 60
ATGCTTTTAG TGGTCAAGTC CGATGCTAGT GTAGAGCTGC CTAACACTACT AATTGCGGT 120
TACTTACGAG TGTCAGGACG TGGGGAGGTG ACGTGTGCA ACCGTGAGGA ATTAACAAGA 180
GATTTTGAGG GCAATCATCA TACGGGTGAT CGTTCTAGAA TCATACAATA TGACAGCGAG 240
TCTGCTTTTG AGGAATTCAA CAACTCTGAT TCGTAGTGA AGTTTTTCCT AGAGACTGGT 300
AGTGTCTTTT GGTTTTTCTT TCGAAGTGAA ACCAAAGGTA GAGCGGTGCG ACATTTGCGC 360
ACCTTCTTCG AAGCTAACAA TTTCTTCTTT GGATCGCATT GCGGTACCAT GGAGTATTGT 420
TTGAAGCAGG TACTAACTGA AACTGAATCT ATAATCGATT CTTTTTGCGA AGAAAGAAAT 480
CGTTAA 486

The second undefined grapevine leafroll virus protein or polypeptide has a deduced amino acid sequence corresponding to SEQ. ID. No. 17 as follows:

Met Glu Asp Tyr Glu Glu Lys Ser Glu Ser Leu Ile Leu Leu Arg Thr
1 5 10 15
Asn Leu Asn Thr Met Leu Leu Val Val Lys Ser Asp Ala Ser Val Glu
20 25 30
Leu Pro Lys Leu Leu Ile Cys Gly Tyr Leu Arg Val Ser Gly Arg Gly
35 40 45
Glu Val Thr Cys Cys Asn Arg Glu Glu Leu Thr Arg Asp Phe Glu Gly
50 55 60
Asn His His Thr Val Ile Arg Ser Arg Ile Ile Gln Tyr Asp Ser Glu
65 70 75 80
Ser Ala Phe Glu Glu Phe Asn Asn Ser Asp Cys Val Val Lys Phe Phe
85 90 95
Leu Glu Thr Gly Ser Val Phe Trp Phe Phe Leu Arg Ser Glu Thr Lys
100 105 110

Gly Arg Ala Val Arg His Leu Arg Thr Phe Phe Glu Ala Asn Asn Phe
115 120 125
Phe Phe Gly Ser His Cys Gly Thr Met Glu Tyr Cys Leu Lys Gln Val
130 135 140
Leu Thr Glu Thr Glu Ser Ile Ile Asp Ser Phe Cys Glu Glu Arg Asn
145 150 155 160
Arg

and a molecular weight from about 17 to about 21 kDa, preferably about 19 kDa.

Yet another such DNA molecule (GLRaV-2 ORF8) includes nucleotides 14667-15284 of SEQ. ID. No. 1 and codes for a third undefined grapevine leafroll virus protein or polypeptide. This DNA molecule comprises a nucleotide sequence corresponding to SEQ. ID. No. 18 as follows:

ATGAGGGTTA TAGTGCTCC TTATGAAGCT GAAGACATTC TGAAAGATC GACTGACATG 60
TTACGAAACA TAGACAGTGG GGTCTTGAGC ACTAAAGAAT GTATCAAGGC ATTCTCGAGC 120
ATAACGCGAG ACCTACATTG TGCGAAGGCT TCCTACCACT GGGGTGTGTA CACTGGGTGA 180
TATCAGCGTA ATTGCGCTGA AAAACGTTTA ATTGACACGG TGGAGTCAAA CATACGGTTG 240
GCTCAACCTC TCGTGCGTGA AAAAGTGGCG GTTCATTTTT GTAAGGATGA ACCAAAAGAG 300
CTAGTAGCAT TCATCACGCG AAAGTACGTG GAACTCACGG GCGTGGGAGT GAGAGAAGCG 360
GTGAAGAGGG AAATGCGCTC TCTTACCAAA ACAGTTTTAA ATAAAATGTC TTTGGAATG 420
GCGTTTTACA TGTCACCACG AGCGTGGAAG AACGCTGAAT GGTTAGAAT AAAATTTTCA 480
CCTGTGAAAA TCTTTAGAGA TCTGCTATTA GACGTGGAAG CGCTCAACGA ATTGTGCGCC 540
GAAGATGATG TTCACGTCGA CAAAGTAAAT GAGAATGGGG ACGAAAATCA CGACCTCGAA 600
CTCCAAGACG AATGTTAA 618

The third undefined protein or polypeptide has a deduced amino acid sequence corresponding to SEQ. ID. No. 19 as follows:

Met Arg Val Ile Val Ser Pro Tyr Glu Ala Glu Asp Ile Leu Lys Arg
1 5 10 15
Ser Thr Asp Met Leu Arg Asn Ile Asp Ser Gly Val Leu Ser Thr Lys
20 25 30
Glu Cys Ile Lys Ala Phe Ser Thr Ile Thr Arg Asp Leu His Cys Ala
35 40 45

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Lys Ala Ser Tyr Gln Trp Gly Val Asp Thr Gly Leu Tyr Gln Arg Asn
50 55 60

Cys Ala Glu Lys Arg Leu Ile Asp Thr Val Glu Ser Asn Ile Arg Leu
65 70 75 80

Ala Gln Pro Leu Val Arg Glu Lys Val Ala Val His Phe Cys Lys Asp
85 90 95

Glu Pro Lys Glu Leu Val Ala Phe Ile Thr Arg Lys Tyr Val Glu Leu
100 105 110

Thr Gly Val Gly Val Arg Glu Ala Val Lys Arg Glu Met Arg Ser Leu
115 120 125

Thr Lys Thr Val Leu Asn Lys Met Ser Leu Glu Met Ala Phe Tyr Met
130 135 140

Ser Pro Arg Ala Trp Lys Asn Ala Glu Trp Leu Glu Leu Lys Phe Ser
145 150 155 160

Pro Val Lys Ile Phe Arg Asp Leu Leu Leu Asp Val Glu Thr Leu Asn
165 170 175

Glu Leu Cys Ala Glu Asp Asp Val His Val Asp Lys Val Asn Glu Asn
180 185 190

Gly Asp Glu Asn His Asp Leu Glu Leu Gln Asp Glu Cys
195 200 205

and a molecular weight from about 22 to about 26 kDa, preferably about 24 kDa.

Another DNA molecule of the present invention (GLRaV-2 3' UTR) includes nucleotides 15285-15500 of SEQ. ID. No. 1 and comprises a nucleotide sequence corresponding to SEQ. ID. No. 23 as follows:

ACATTGGTTA AGTTTAACGA AATGATTAG TAAATAATAA ATCGAACGTG GGTGTATCTA	60
CCTGACGTAT CAACTTAAGC TGTTACTGAG <u>TAATTAAACC</u> AACAAAGTGT <u>GGTGAATGT</u>	120
GTATGTTGAT GTAGAGAAAA ATCCGTTTGT AGAACGGTGT TTTTCTCTTC TTTATTTT	180
AAAAAAAAAT AAAAAAAAAA AAAAAAAGC GGCCGC	216

- 5 Also encompassed by the present invention are fragments of the DNA molecules of the present invention. Suitable fragments capable of imparting grapevine leafroll resistance to grape plants are constructed by using appropriate restriction sites, revealed by inspection of the DNA molecule's sequence, to: (i) insert an interposon (Felley et al., "Interposon Mutagenesis of Soil and Water Bacteria: a Family of DNA
- 10 Fragments Designed for in vitro Insertion Mutagenesis of Gram-negative Bacteria," Gene, 52:147-15 (1987), which is hereby incorporated by reference) such that truncated

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forms of the grapevine leafroll virus coat polypeptide or protein, that lack various amounts of the C-terminus, can be produced or (ii) delete various internal portions of the protein. Alternatively, the sequence can be used to amplify any portion of the coding region, such that it can be cloned into a vector supplying both transcription and translation start signals.

Suitable DNA molecules are those that hybridize to a DNA molecule comprising a nucleotide sequence of at least 15 continuous bases of SEQ. ID. No. 1 under stringent conditions characterized by a hybridization buffer comprising 0.9M sodium citrate ("SSC") buffer at a temperature of 37°C and remaining bound when subject to washing with SSC buffer at 37°C; and preferably in a hybridization buffer comprising 20% formamide in 0.9M saline/0.9M SSC buffer at a temperature of 42°C and remaining bound when subject to washing at 42°C with 0.2x SSC buffer at 42°C.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of nucleotides that have minimal influence on the properties, secondary structure and hydropathic nature of the encoded polypeptide. For example, the nucleotides encoding a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The nucleotide sequence may also be altered so that the encoded polypeptide is conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The protein or polypeptide of the present invention is preferably produced in purified form (preferably, at least about 80%, more preferably 90%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is isolated by ~~lysing and sonication~~. After washing, the lysate pellet is resuspended in buffer containing Tris-HCl. During dialysis, a precipitate forms from this protein solution. The solution is centrifuged, and the pellet is washed and resuspended in the buffer containing Tris-HCl. Proteins are resolved by electrophoresis through an SDS 12% polyacrylamide gel.

The DNA molecule encoding the grapevine leafroll virus (type 2) protein or polypeptide of the present invention can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the

necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC184, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology, vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1982), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria or transformed via particle bombardment (i.e. biolistics). The expression elements of these vectors vary in their

strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA ("mRNA") translation).

Transcription of DNA is dependent upon the presence of a promoter which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promoters differ from those of procaryotic promoters. Furthermore, eucaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promoters are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P_R and P_L promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5* (*tac*) promoter or other *E. coli* promoters produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operons, the addition of specific inducers is necessary for efficient transcription of the inserted DNA.

For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires a Shine-Dalgarno ("SD") sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecules encoding the various grapevine leafroll virus (type 2) proteins or polypeptides, as described above, have been cloned into an expression system, they are ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The present invention also relates to RNA molecules which encode the various grapevine leafroll virus (type 2) proteins or polypeptides described above. The transcripts can be synthesized using the host cells of the present invention by any of the conventional techniques. The mRNA can be translated either *in vitro* or *in vivo*. Cell-free systems typically include wheat-germ or reticulocyte extracts. *In vivo* translation can be effected, for example, by microinjection into frog oocytes.

One aspect of the present invention involves using one or more of the above DNA molecules encoding the various proteins or polypeptides of a grapevine leafroll virus (type 2) to transform grape plants in order to impart grapevine leafroll resistance to the plants. The mechanism by which resistance is imparted is not known. In one hypothetical mechanism, the transformed plant can express a protein or polypeptide of grapevine leafroll virus (type 2), and, when the transformed plant is inoculated by a

grapevine leafroll virus, such as GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-4, GLRaV-5, or GLRaV-6, or combinations of these, the expressed protein or polypeptide prevents translation of the viral DNA.

In this aspect of the present invention the subject DNA molecule
5 incorporated in the plant can be constitutively expressed. Alternatively, expression can be regulated by a promoter which is activated by the presence of grapevine leafroll virus. Suitable promoters for these purposes include those from genes expressed in response to grapevine leafroll virus infiltration.

The isolated DNA molecules of the present invention can be utilized to
10 impart grapevine leafroll virus resistance for a wide variety of grapevine plants. The DNA molecules are particularly well suited to imparting resistance to *Vitis* scion or rootstock cultivars. Scion cultivars which can be protected include those commonly referred to as Table or Raisin Grapes, such as Alden, Almeria, Anab-E-Shahi, Autumn Black, Beauty Seedless, Black Corinth, Black Damascus, Black Malvoisie, Black Prince,
15 Blackrose, Bronx Seedless, Burgrave, Calmeria, Campbell Early, Canner, Cardinal, Catawba, Christmas, Concord, Dattier, Delight, Diamond, Dizmar, Duchess, Early Muscat, Emerald Seedless, Emperor, Exotic, Ferdinand de Lesseps, Fiesta, Flame seedless, Flame Tokay, Gasconade, Gold, Himrod, Hunisa, Hussiene, Isabella, Italia, July Muscat, Khandahar, Katta, Kourgane, Kishmishi, Loose Perlette, Malaga, Monukka,
20 Muscat of Alexandria, Muscat Flame, Muscat Hamburg, New York Muscat, Niabell, Niagara, Olivette blanche, Ontario, Pierce, Queen, Red Malaga, Ribier, Rish Baba, Romulus, Ruby Seedless, Schuyler, Seneca, Suavis (IP 365), Thompson seedless, and Thomuscat. They also include those used in wine production, such as Aleatico, Alicante Bouschet, Aligote, Alvarelhao, Aramon, Baco blanc (22A), Burger, Cabernet franc,
25 Cabernet, Sauvignon, Calzin, Carignane, Charbono, Chardonnay, Chasselas dore, Chenin blanc, Clairette blanche, Early Burgundy, Emerald Riesling, Feher Szagos, Fernao Pires, Flora, French Colombard, Fresia, Furmint, Gamay, Gewurztraminer, Grand noir, Gray Riesling, Green Hungarian, Green Veltliner, Grenache, Grillo, Helena, Inzolia, Lagrein, Lambrusco de Salamino, Malbec, Malvasia bianca, Mataro, Melon, Merlot, Meunier,
30 Mission, Montua de Pilas, Muscadelle du Bordelais, Muscat blanc, Muscat Ottonel, Muscat Saint-Vallier, Nebbiolo, Nebbiolo fino, Nebbiolo Lampia, Orange Muscat, Palomino, Pedro Ximenes, Petit Bouschet, Petite Sirah, Peverella, Pinot noir, Pinot Saint-George, Primitivo di Gioia, Red Veltliner, Refosco, Rkatsiteli, Royalty, Rubired, Ruby Cabernet, Saint-Emilion, Saint Macaire, Salvador, Sangiovese, Sauvignon blanc,

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Sauvignon gris, Sauvignon vert, Scarlet, Seibel 5279, Seibel 9110, Seibel 13053, Semillon, Servant, Shiraz, Souzao, Sultana Crimson, Sylvaner, Tannat, Teroldico, Tinta Madeira, Tinto cao, Touriga, Traminer, Trebbiano Toscano, Trousseau, Valdepenas, Viognier, Walschriesling, White Riesling, and Zinfandel. Rootstock cultivars which can be protected include Couderc 1202, Couderc 1613, Couderc 1616, Couderc 3309, Dog Ridge, Foex 33 EM, Freedom, Ganzin 1 (A x R #1), Harmony, Kober 5BB, LN33, Millardet & de Grasset 41B, Millardet & de Grasset 420A, Millardet & de Grasset 101-14, Oppenheim 4 (SO4), Paulsen 775, Paulsen 1045, Paulsen 1103, Richter 99, Richter 110, Riparia Gloire, Ruggeri 225, Saint-George, Salt Creek, Teleki 5A, Vitis rupestris Constantia, *Vitis californica*, and *Vitis girdiana*.

There exists an extensive similarity in the hsp70-related sequence regions of GLRaV-2 and other closteroviruses, such as tristeza virus and beet yellows virus. Consequently, the GLRaV-2 hsp70-related gene can also be used to produce transgenic plants or cultivars other than grape, such as citrus or sugar beet, which are resistant to closteroviruses other than grapevine leafroll, such as tristeza virus and beet yellows virus.

Suitable citrus cultivars include lemon, lime, orange, grapefruit, pineapple, tangerine, and the like, such as Joppa, Maltaise Ovale, Parson (Parson Brown), Pera, Pineapple, Queen, Shamouti, Valencia, Tenerife, Imperial Doblefina, Washington Sanguine, Moro, Sanguinello Moscato, Spanish Sanguinelli, Tarocco, Atwood, Australian, Bahia, Baiana, Cram, Dalmau, Eddy, Fisher, Frost Washington, Gillette, LengNavelina, Washington, Satsuma Mandarin, Dancy, Robinson, Ponkan, Duncan, Marsh, Pink Marsh, Ruby Red, Red Seedless, Smooth Seville, Orlando Tangelo, Eureka, Lisbon, Meyer Lemon, Rough Lemon, Sour Orange, Persian Lime, West Indian Lime, Bearss, Sweet Lime, Troyer Citrange, and Citrus Trifoliata. Each of these citrus cultivars is suitable for producing transgenic citrus plants resistant to tristeza virus.

The economically important species of sugar beet is *Beta vulgaris* L., which has four important cultivar types: sugar beet, table beet, fodder beet, and Swiss chard. Each of these beet cultivars is suitable for producing transgenic beet plants resistant to beet yellows virus, as described above.

Because GLRaV-2 has been known to infect tobacco plants (e.g., *Nicotiana benthamiana*), it is also desirable to produce transgenic tobacco plants which are resistant to grapevine leafroll viruses, such as GLRaV-2.

Plant tissue suitable for transformation include leaf tissue, root tissue, meristems, zygotic and somatic embryos, and anthers. It is particularly preferred to utilize embryos obtained from anther cultures.

The expression system of the present invention can be used to transform virtually any plant tissue under suitable conditions. Tissue cells transformed in accordance with the present invention can be grown *in vitro* in a suitable medium to impart grapevine leafroll virus resistance. Transformed cells can be regenerated into whole plants such that the protein or polypeptide imparts resistance to grapevine leafroll virus in the intact transgenic plants. In either case, the plant cells transformed with the recombinant DNA expression system of the present invention are grown and caused to express that DNA molecule to produce one of the above-described grapevine leafroll virus proteins or polypeptides and, thus, to impart grapevine leafroll virus resistance.

In producing transgenic plants, the DNA construct in a vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

One technique of transforming plants with the DNA molecules in accordance with the present invention is by contacting the tissue of such plants with an inoculum of a bacteria transformed with a vector comprising a gene in accordance with the present invention which imparts grapevine leafroll resistance. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Bacteria from the genus *Agrobacterium* can be utilized to transform plant cells. Suitable species of such bacterium include *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes*. *Agrobacterium tumefaciens* (e.g., strains C58, LBA4404, or EHA105) is particularly useful due to its well-known ability to transform plants.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by *Agrobacterium* and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. I: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally ~~2~~ suspension of transformed protoplasts or a petri plate containing explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure so that the DNA construct is present in the resulting plants. Alternatively, transgenic seeds are recovered from the transgenic plants. These seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants.

Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., and in Emershad et al., "Somatic Embryogenesis and Plant Development from Immature Zygotic Embryos of Seedless Grapes (*Vitis vinifera*)," Plant Cell Reports, 14:6-12 (1995) ("Emershad (1995)"), which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under

conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA.

Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Once a grape plant tissue, citrus plant tissue, beet plant tissue, or tobacco plant tissue is transformed in accordance with the present invention, the transformed tissue is regenerated to form a transgenic plant. Generally, regeneration is accomplished by culturing transformed tissue on medium containing the appropriate growth regulators and nutrients to allow for the initiation of shoot meristems. Appropriate antibiotics are added to the regeneration medium to inhibit the growth of *Agrobacterium* and to select for the development of transformed cells. Following shoot initiation, shoots are allowed to develop tissue culture and are screened for marker gene activity.

The DNA molecules of the present invention can be made capable of transcription to a messenger RNA, which, although encoding for a grapevine leafroll virus (type 2) protein or polypeptide, does not translate to the protein. This is known as RNA-mediated resistance. When a *Vitis* scion or rootstock cultivar, or a citrus, beet, or tobacco cultivar, is transformed with such a DNA molecule, the DNA molecule can be transcribed under conditions effective to maintain the messenger RNA in the plant cell at low level density readings. Density readings of between 15 and 50 using a Hewlett ScanJet and Image Analysis Program are preferred.

A portion of one or more DNA molecules of the present invention as well as other DNA molecules can be used in a transgenic grape plant, citrus plant, beet plant, or tobacco plant in accordance with U.S. Patent Application Serial No. 09/025,635, which is hereby incorporated herein by reference.

The grapevine leafroll virus (type 2) protein or polypeptide of the present invention can also be used to raise antibodies or binding portions thereof or probes. The antibodies can be monoclonal or polyclonal.

Monoclonal antibody production may be effected by techniques which are well-known in the art. Basically, the process involves first obtaining immune cells (lymphocytes) from the spleen of a mammal (e.g., mouse) which has been previously immunized with the antigen of interest either *in vivo* or *in vitro*. The antibody-secreting lymphocytes are then fused with (mouse) myeloma cells or transformed cells, which are

capable of replicating indefinitely in cell culture, thereby producing an immortal, immunoglobulin-secreting cell line. The resulting fused cells, or hybridomas, are cultured, and the resulting colonies screened for the production of the desired monoclonal antibodies. Colonies producing such antibodies are cloned, and grown either *in vivo* or *in vitro* to produce large quantities of antibody. A description of the theoretical basis and practical methodology of fusing such cells is set forth in Kohler and Milstein, Nature, 256:495 (1975), which is hereby incorporated by reference.

Mammalian lymphocytes are immunized by *in vivo* immunization of the animal (e.g., a mouse) with the protein or polypeptide of the present invention. Such immunizations are repeated as necessary at intervals of up to several weeks to obtain a sufficient titer of antibodies. Following the last antigen boost, the animals are sacrificed and spleen cells removed.

Fusion with mammalian myeloma cells or other fusion partners capable of replicating indefinitely in cell culture is effected by standard and well-known techniques, for example, by using polyethylene glycol ("PEG") or other fusing agents. (See Milstein and Kohler, Eur. J. Immunol., 6:511 (1976), which is hereby incorporated by reference.) This immortal cell line, which is preferably murine, but may also be derived from cells of other mammalian species, including but not limited to rats and humans, is selected to be deficient in enzymes necessary for the utilization of certain nutrients, to be capable of rapid growth, and to have good fusion capability. Many such cell lines are known to those skilled in the art, and others are regularly described.

Procedures for raising polyclonal antibodies are also well known. Typically, such antibodies can be raised by administering the protein or polypeptide of the present invention subcutaneously to New Zealand white rabbits which have first been bled to obtain pre-immune serum. The antigens can be injected at a total volume of 100 µl per site at six different sites. Each injected material will contain synthetic surfactant adjuvant pluronic polyols, or pulverized acrylamide gel containing the protein or polypeptide after SDS-polyacrylamide gel electrophoresis. The rabbits are then bled two weeks after the first injection and periodically boosted with the same antigen three times every six weeks. A sample of serum is then collected 10 days after each boost. Polyclonal antibodies are then recovered from the serum by affinity chromatography using the corresponding antigen to capture the antibody. Ultimately, the rabbits are euthenized with pentobarbital 150 mg/Kg IV. This and other procedures for raising

polyclonal antibodies are disclosed in Harlow et. al., editors, Antibodies: A Laboratory Manual (1988), which is hereby incorporated by reference.

In addition to utilizing whole antibodies, binding portions of such antibodies can be used. Such binding portions include Fab fragments, F(ab')₂ fragments, and Fv fragments. These antibody fragments can be made by conventional procedures, such as proteolytic fragmentation procedures, as described in Goding, Monoclonal Antibodies: Principles and Practice, New York:Academic Press, pp. 98-118 (1983), which is hereby incorporated by reference.

The present invention also relates to probes found either in nature or prepared synthetically by recombinant DNA procedures or other biological procedures. Suitable probes are molecules which bind to grapevine leafroll (type 2) viral antigens identified by the monoclonal antibodies of the present invention. Such probes can be, for example, proteins, peptides, lectins, or nucleic acid probes.

The antibodies or binding portions thereof or probes can be administered to grapevine leafroll virus infected scion cultivars or rootstock cultivars. Alternatively, at least the binding portions of these antibodies can be sequenced, and the encoding DNA synthesized. The encoding DNA molecule can be used to transform plants together with a promoter which causes expression of the encoded antibody when the plant is infected by grapevine leafroll virus. In either case, the antibody or binding portion thereof or probe will bind to the virus and help prevent the usual leafroll response.

Antibodies raised against the GLRaV-2 proteins or polypeptides of the present invention or binding portions of these antibodies can be utilized in a method for detection of grapevine leafroll virus in a sample of tissue, such as tissue (e.g., scion or rootstock) from a grape plant or tobacco plant. Antibodies or binding portions thereof suitable for use in the detection method include those raised against a helicase, a methyltransferase, a papain-like protease, an RNA-dependent RNA polymerase, a heat shock 70 protein, a heat shock 90 protein, a coat protein, a diverged coat protein, or other proteins or polypeptides in accordance with the present invention. Any reaction of the sample with the antibody is detected using an assay system which indicates the presence of grapevine leafroll virus in the sample. A variety of assay systems can be employed, such as enzyme-linked immunosorbent assays, radioimmunoassays, gel diffusion precipitin reaction assays, immunodiffusion assays, agglutination assays, fluorescent immunoassays, protein A immunoassays, or immunoelectrophoresis assays.

Alternatively, grapevine leafroll virus can be detected in such a sample using a nucleotide sequence of the DNA molecule, or a fragment thereof, encoding for a protein or polypeptide of the present invention. The nucleotide sequence is provided as a probe in a nucleic acid hybridization assay or a gene amplification detection procedure (e.g., using a polymerase chain reaction procedure). The nucleic acid probes of the present invention may be used in any nucleic acid hybridization assay system known in the art, including, but not limited to, Southern blots (Southern, E.M., "Detection of Specific Sequences Among DNA Fragments Separated by Gel Electrophoresis," J. Mol. Biol., 98:503-17 (1975), which is hereby incorporated by reference), Northern blots (Thomas, P.S., "Hybridization of Denatured RNA and Small DNA Fragments Transferred to Nitrocellulose," Proc. Nat'l Acad. Sci. USA, 77:5201-05 (1980), which is hereby incorporated by reference), and Colony blots (Grunstein, M., et al., "Colony Hybridization: A Method for the Isolation of Cloned cDNAs that Contain a Specific Gene," Proc. Nat'l Acad. Sci. USA, 72:3961-65 (1975), which is hereby incorporated by reference). Alternatively, the probes can be used in a gene amplification detection procedure (e.g., a polymerase chain reaction). Erlich, H.A., et. al., "Recent Advances in the Polymerase Chain Reaction," Science 252:1643-51 (1991), which is hereby incorporated by reference. Any reaction with the probe is detected so that the presence of a grapevine leafroll virus in the sample is indicated. Such detection is facilitated by providing the probe of the present invention with a label. Suitable labels include a radioactive compound, a fluorescent compound, a chemiluminescent compound, an enzymatic compound, or other equivalent nucleic acid labels.

Depending upon the desired scope of detection, it is possible to utilize probes having nucleotide sequences that correspond with conserved or variable regions of the ORF or UTR. For example, to distinguish a grapevine leafroll virus from other related viruses (e.g., other closteroviruses), it is desirable to use probes which contain nucleotide sequences that correspond to sequences more highly conserved among all grapevine leafroll viruses. Also, to distinguish between different grapevine leafroll viruses (i.e., GLRaV-2 from GLRaV-1, GLRaV-3, GLRaV-4, GLRaV-5, and GLRaV-6), it is desirable to utilize probes containing nucleotide sequences that correspond to sequences less highly conserved among the different grapevine leafroll viruses.

Nucleic acid (DNA or RNA) probes of the present invention will hybridize to complementary GLRaV-2 nucleic acid under stringent conditions. Generally, stringent conditions are selected to be about 50°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under

defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. The T_m is dependent upon the solution conditions and the base composition of the probe, and may be calculated using the following equation:

$$\begin{aligned} T_m = & 79.8^{\circ}\text{C} + (18.5 \times \text{Log}[\text{Na}^+]) \\ & + (58.4^{\circ}\text{C} \times \%[\text{G}+\text{C}]) \\ & - (820 / \# \text{bp in duplex}) \\ & - (0.5 \times \% \text{ formamide}) \end{aligned}$$

Nonspecific binding may also be controlled using any one of a number of known techniques such as, for example, blocking the membrane with protein-containing solutions, addition of heterologous RNA, DNA, and SDS to the hybridization buffer, and treatment with RNase. Wash conditions are typically performed at or below stringency. Generally, suitable stringent conditions for nucleic acid hybridization assays or gene amplification detection procedures are as set forth above. More or less stringent conditions may also be selected.

EXAMPLES

The following examples are provided to illustrate embodiments of the present invention but are by no means intended to limit its scope.

Example 1 - Northern Hybridization

Specificity of the selected clones was confirmed by Northern hybridization. Northern hybridization was performed after electrophoresis of the dsRNA of GLRaV-2 in 1% agarose non-denaturing condition gel. The agarose gel was denatured by soaking in 50 mM NaOH containing 0.4 M NaCl for 30 min, and then neutralized with 0.1 M Tris-HCl (pH 7.5) containing 0.5 M NaCl for another 30 min. RNA was sandwich blotted overnight onto Genescreen™ plus membrane (Dupont NEN Research Product) in 10 X SSC buffer and hybridized as described by the manufacturer's instructions (DuPont, NEN).

Example 2 - Sequencing and Computer Assisted Nucleotide and Amino Acid Sequence Analysis

DNA inserts were sequenced in pBluescript SK+ by using T3 and T7 universal primers for the terminal region sequence and additional oligonucleotide primers

designed according to the known sequence for the internal region sequence. Purification of plasmid DNA was performed by a modified mini alkaline-lysis/PEG precipitation procedure described by the manufacturer (Applied Biosystems, Inc.). Nucleotide sequencing was performed on both strands of cDNA by using ABI TaqDyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Inc.). Automatic sequencing was performed on an ABI373 Automated Sequencer (Applied Biosystems, Inc.) at Cornell University, Geneva, NY.

The nucleotide sequences of GLRaV-2 were assembled and analyzed with the programs of EditSeq and SeqMan, respectively, of DNASTAR package (Madison, WI). Amino acid sequences deduced from nucleotide sequences and its encoding open reading frames were conducted using the MapDraw program. Multiple alignments of amino acid sequences, identification of consensus amino acid sequences, and generation of phylogenetic trees were performed using the Clustal method in the MegAlign program. The nucleotide and amino acid sequences of other closteroviruses were obtained with the Entrez Program; and sequence comparisons with nonredundant databases were searched with the Blast Program from the National Center for Biotechnology Information.

Example 3 - Isolation of dsRNA

Several vines of GLRaV-2 infected *Vitis vinifera* cv Pinot Noir that originated from a central New York vineyard served as the source for dsRNA isolation and cDNA cloning. dsRNA was extracted from phloem tissue of infected grapevines according to the method described by Hu et al., "Characterization of Closterovirus-Like Particles Associated with Grapevine Leafroll Disease," J. Phytopathology 128:1-14 (1990), which is hereby incorporated by reference. Purification of the high molecular weight dsRNA (ca 15 kb) was carried out by electrophoretic separation of the total dsRNA on a 0.7% low melting point agarose gel and extraction by phenol/chloroform following the method described by Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, New York (1989), which is hereby incorporated by reference. Concentration of dsRNA was estimated with UV fluorescent density of an ethidium bromide stained dsRNA band in comparison with a known concentration of DNA marker.

Example 4 - cDNA Synthesis and Cloning

cDNA synthesis was performed following the method initially described by Jelkmann et al., "Cloning of Four Plant Viruses From Small Quantities of Double-Stranded RNA," Phytopathology 79:1250-53 (1989) and modified by Ling et al., "The Coat Protein Gene of Grapevine Leafroll Associated Closterovirus-3: Cloning, Nucleotide Sequencing and Expression in Transgenic Plants," Arch. Virology 142:1101-16 (1997), both of which are hereby incorporated by reference. About 100 ng of high molecular weight dsRNA purified from low melting agarose gel was denatured in 20 mM methylmercuric hydroxide and incubated at room temperature for 10 min with 350 ng of random primers. First strand cDNA was synthesized by using avian myeloblastosis virus (AMV) reverse transcriptase. Second strand cDNA was obtained by using RNase H and *E.coli* DNA polymerase I. Double-stranded cDNA was blunt ended with T4 DNA polymerase and ligated with EcoR I adapters. The cDNA, which had EcoR I adapters at the ends, was activated by kinase reaction and ligated into Lambda ZAP II/EcoR I prepared arms following the manufacturer's instruction (Stratagene). The recombinant DNA was then packaged *in vitro* to Gigapack® II packaging extract (Stratagene). The packaged phage particles were amplified and titered according to the manufacturer's instruction.

Two kinds of probes were used to identify GLRaV-2 specific clones from the library. One type was prepared from the synthesized cDNA that was amplified by PCR after ligation to the specific EcoR I Uni-Amp™ adapters (Clontech); and the other type was DNA inserts or PCR products from already sequenced clones. Clones from the cDNA library were selected by colony-lifting hybridization onto the colony/plaque Screen membrane (NEN Research Product) with the probe described above. The probe was prepared by labeling with ³²P [α -dATP] using Klenow fragment of *E.coli* DNA polymerase I. Prehybridization, hybridization, and washing steps were carried out at 65°C according to the manufacturer's instruction (Dupont, NEN Research Product). Selected plaques were converted to recombinant pBluescript by *in vivo* excision method according to the manufacturer's instruction (Stratagene).

To obtain clones representing the extreme 3'-terminus of GLRaV-2, dsRNA was polyadenylated by yeast poly(A) polymerase. Using poly(A)-tailed dsRNA as template, cDNA was amplified by RT-PCR with oligo(dT)18 and a specific primer, CP-1/T7R, which

is derived from the clone CP-1 and has a nucleotide sequence according to SEQ. ID. No. 20 as follows:

TGCTGGAGCT TGAGGTTCTG C

21

The resulting PCR product (3'-PCR) was cloned into a TA vector (Invitrogen) and sequenced.

As shown in Figure 1A, a high molecular weight dsRNA of ca. 15 kb was consistently identified from GLRaV-2 infected grapevines, but not from healthy vines. In addition, several low molecular weight dsRNAs were also detected from infected tissue. The yield of dsRNA of GLRaV-2 was estimated between 5-10 ng/15 g phloem tissue, which was much lower than that of GLRaV-3 (Hu et al., "Characterization of Closterovirus-Like Particles Associated with Grapevine Leafroll Disease," *J. Phytopathology* 128:1-14 (1990), which is hereby incorporated by reference). Only the high molecular weight dsRNA that was purified from low melting point agarose gel was used for cDNA synthesis, cloning and establishment of the Lambda/ZAP II cDNA library.

Two kinds of probes were used for screening the cDNA library. The initial clones were identified by hybridization with Uni-Amp™ PCR-amplified cDNA as probes. The specificity of these clones (e.g., TC-1) ranging from 200 to 1,800 bp in size was confirmed by Northern hybridization to dsRNA of GLRaV-2 as shown in Figure 1B. Additionally, over 40 different clones ranging from 800 to 7,500 bp in size were identified following hybridization with the probes generated from GLRaV-2 specific cDNA clones or from PCR products. Over 40 clones were then sequenced on the both strands (Figure 2).

Example 5 - Expression of the Coat Protein in *E. coli* and Immunoblotting

To determine that ORF6 was the coat protein gene of GLRaV-2, the complete ORF6 DNA molecule was subcloned from a PCR product and inserted into the fusion protein expression vector pMAL-C2 (New England Biolabs, Inc.). The specific primers used for the PCR reaction were CP-96F and CP-96R, in which an EcoR I or BamH I site was included to facilitate cloning. CP-96F was designed to include the start codon of the CP and comprises a nucleotide sequence according to SEQ. ID. NO. 21 as follows:

CGGAATTCAC CATGGAGTTG ATGTCCGACA G

31

CP-96R was 66 nucleotides downstream of the stop codon of the CP and comprises the nucleotide sequence corresponding to SEQ. ID. No. 22 as follows:

AGCGGATCCA TGGCAGATTC GTGCGTAGCA GTA

33

The coat protein was expressed as a fusion protein with maltose binding protein (MBP) of *E. coli* under the control of a "tac" promoter and suppressed by the "lac" repressor. The MBP-CP fusion protein was induced by adding 0.3 mM isopropyl- β -D-thio-galactopyranoside (IPTG) and purified by a one step affinity column according to the manufacturer's instruction (New England, Biolabs, Inc.). The MBP-CP fusion protein or the coat protein cleaved from the fusion protein was tested to react with specific antiserum of GLRaV-2 (kindly provided by Dr. Charles Greif of INRA, Colmar, France) on Western blot according to the method described by Hu et al., "Characterization of Closterovirus-Like Particles Associated with Grapevine Leafroll Disease," *J. Phytopathology* 128:1-14 (1990), which is hereby incorporated by reference. In contrast, the non-recombinant plasmids or uninduced cells did not react to the antiserum of GLRaV-2.

Example 6 - Sequence Analysis and Genome Organization of GLRaV-2

A total of 15,500 bp of the RNA genome of GLRaV-2 was sequenced and deposited in GenBank (accession number AF039204). About 85% of the total RNA genome was revealed from at least two different clones. The sequence in the coat protein gene region was determined and confirmed from several different overlapping clones. The genome organization of GLRaV-2, shown in Figure 2, includes nine open reading frames (e.g., ORF1a, 1b-8).

ORF1a and ORF1b: Analysis of the amino acid sequence of the N-terminal portion of GLRaV-2 ORF1a encoded product revealed two putative papain-like protease domains, which showed significant similarity to the papain-like leader protease of BYV (Agranovsky et al., "Beet Yellows Closterovirus: Complete Genome Structure and Identification of a Papain-like Thiol Protease," *Virology* 198:311-24 (1994), which is hereby incorporated by reference). Thus, it allowed prediction of the catalytic cysteine and histidine residues for the putative GLRaV-2 protease. Upon alignment of the sequence of the papain-like protease of BYV with that of GLRaV-2, the cleavage site at residues Gly-Gly (amino acid 588-589) of BYV aligned with the corresponding alanine-glycine (Ala-Gly) and Gly-Gly dipeptide of GLRaV-2 (Figure 3A). Cleavage at this site would result in a leader protein and

a 234 kDa (2090 amino acid) C-terminal fragment consisting of MT and HEL domains. However, the region upstream of the papain-like protease domain in GLRaV-2 did not show similarity to the corresponding region of BYV. In addition, variability in the residues located at the scissile bond (Gly in the BYV and Ala in the GLRaV-2) was present. Similar
5 variability of the cleavage site residue in the P-PRO domain has been described in LChV (Jelkmann et al., "Complete Genome Structure and Phylogenetic Analysis of Little Cherry Virus, a Mealybug-Transmissible Closterovirus. J. General Virology 78:2067-71 (1997), which is hereby incorporated by reference).

Database searching with the deduced amino acid sequence of the ORF1a/1b
10 encoded protein revealed a significant similarity to the MT, HEL and RdRP domains of the other closteroviruses. The region downstream of the P-PRO cleavage site showed a significant similarity (57.4% identity in a 266-residues alignment) to the putative methyltransferase domain of BYV and contained all the conserved motifs typical of positive-strand RNA viral type I MTs (Figure 3B). The C-terminal portion of the ORF1a was
15 identified as a helicase domain, the sequence of which showed a high similarity (57.1% identity in a 315-residues alignment) to the helicase domain of BYV and contained the seven conserved motifs characteristic of the Superfamily I helicase of positive-strand RNA viruses (Figure 3C) (Hodgman, "A New Superfamily of Replicative Proteins," Nature 333:22-23 (1988); Koonin and Dolja, "Evolution and Taxonomy of Positive-strand RNA Viruses: Implications of Comparative Analysis of Amino Acid Sequences," Crit. Rev. in Biochem.
20 and Mol. Biol. 28:375-430 (1993), both of which are hereby incorporated by reference).

ORF1b encoded a 460 amino acid polypeptide with a molecular mass of 52,486 Da, counting from the frameshifting site. Database searching with the RdRP showed a significant similarity to the RdRP domains of positive strand RNA viruses. Comparison of
25 the RdRP domains of GLRaV-2 and BYV showed the presence of the eight conserved motifs of RdRP (Figure 3D).

As shown in Figure 8, a tentative phylogenetic tree of the RdRP of GLRaV-2 with respect to other closteroviruses shows that it is closely related to the monopartite closteroviruses BYV, BYSV, and CTV.

30 In closteroviruses, a +1 ribosomal frameshift mechanism has been suggested to be involved in the expression of ORF1b as a large fusion protein with ORF1a (Agranovsky et al., "Beet Yellows Closterovirus: Complete Genome Structure and Identification of a Papain-like Thiol Protease," Virology 198:311-24 (1994); Karasev et al., "Complete Sequence of the Citrus Tristeza Virus RNA Genome," Virology 208:511-20 (1995); Klaassen

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et al., "Genome Structure and Phylogenetic Analysis of Lettuce Infectious Yellows Virus, a Whitefly-Transmitted, Bipartite Closterovirus," Virology 208:99-110 (1995); Karasev et al., "Organization of the 3'-Terminal Half of Beet Yellow Stunt Virus Genome and Implications for the Evolution of Closteroviruses," Virology 221:199-207 (1996); Jelkmann et al.,

5 "Complete Genome Structure and Phylogenetic Analysis of Little Cherry Virus, a Mealybug-Transmissible Closterovirus," J. General Virology 78:2067-71 (1997), all of which are hereby incorporated by reference). In the overlapping ORF1a/1b region of BYV, the slippery sequence of GGGUUUA and two hairpins structure (stem-loop and pseudoknot) are believed to result in a +1 frameshift (Agranovsky et al., "Beet Yellows Closterovirus: Complete

10 Genome Structure and Identification of a Papain-like Thiol Protease," Virology 198:311-24 (1994), which is hereby incorporated by reference). None of these features are conserved in CTV and BYSV (Karasev et al., "Complete Sequence of the Citrus Tristeza Virus RNA Genome," Virology 208:511-20 (1995); Karasev et al., "Organization of the 3'-Terminal Half of Beet Yellow Stunt Virus Genome and Implications for the Evolution of Closteroviruses,"

15 Virology 221:199-207 (1996), both of which are hereby incorporated by reference), in which a ribosomal pausing at a terminator or at a rare codon was suggested to perform the same function. Comparisons of the nucleotide sequence of the C-terminal region of the helicase and the N-terminal region of RdRP of GLRaV-2 with the same region of other closteroviruses revealed a significant similarity to BYV, BYSV, and CTV. As shown in

20 Figure 4, the terminator UAG at the end of C'-terminal helicase of GLRaV-2 aligned with the terminator UAG of BYV and BYSV, and arginine CGG codon of CTV.

ORF2 encodes a small protein consisting of 171 bp (57 amino acid) with a molecular mass of 6,297 Da. As predicted, the deduced amino acid sequence includes a stretch of nonpolar amino acids, which is presumed to form a transmembrane helix. A small

25 hydrophobic analogous protein is also present in BYV, BYSV, CTV, LIYV, and LChV (Agranovsky et al. "Nucleotide Sequence of the 3'-Terminal Half of Beet Yellows Closterovirus RNA Genome Unique Arrangement of Eight Virus Genes," J. General Virology 72:15-24 (1991); Karasev et al., "Organization of the 3'-Terminal Half of Beet Yellow Stunt Virus Genome and Implications for the Evolution of Closteroviruses," Virology

30 221:199-207 (1996); Pappu et al., "Nucleotide Sequence and Organization of Eight 3' Open Reading Frames of the Citrus Tristeza Closterovirus Genome," Virology 199:35-46 (1994); Klaassen et al., "Partial Characterization of the Lettuce Infectious Yellows Virus Genomic RNAs, Identification of the Coat Protein Gene and Comparison of its Amino Acid Sequence With Those of Other Filamentous RNA Plant Viruses," J. General Virology 75:1525-33

(1994); Jelkmann et al., "Complete Genome Structure and Phylogenetic Analysis of Little Cherry Virus, a Mealybug-Transmissible Closterovirus," J. General Virology 78:2067-71 (1997), all of which are hereby incorporated by reference).

ORF3 encodes a 600 amino acid polypeptide with a molecular mass of 65,111

5 Da, which is homologous to the HSP70 cellular heat shock protein. HSP70 is highly conserved among closteroviruses and is probably involved in ATPase activity and the protein to protein interaction for chaperone activity (Agranovsky et al. "The Beet Yellow's Closterovirus p65 Homologue of HSP70 Chaperones has ATPase Activity Associated with its Conserved N-terminal Domain but Interact with Unfolded Protein Chains," J. General Virology 78:535-42 (1997); Agranovsky et al., "Bacterial Expression and Some Properties of the p65, a Homologue of Cell Heat Shock Protein HSP70 Encoded in RNA Genome of Beet Yellow's Closterovirus," Doklady Akademii Nauk. 340:416-18 (1995); Karasev et al., "HSP70-Related 65-kDa Protein of Beet Yellow's Closterovirus is a Microtubule-Binding Protein," FEBS Letters 304:12-14 (1992), all of which are hereby incorporated by reference).

10 As shown in Figure 5, alignment of the complete ORF3 of GLRaV-2 with HSP70 homolog of BYV revealed the presence of the eight conserved motifs. The percentage similarity of the HSP70 between GLRaV-2 and that of BYV, BYSV, CTV, LIYV, and LChV is 47.8%, 47.2%, 38.6%, 20.9%, and 17.7%, respectively.

ORF4 encodes a 551 amino acid protein with a molecular mass of 63,349 Da.

20 Database searching with the ORF4 protein product did not identify similar proteins except those of its counterparts in closteroviruses, BYV (P64), BYSV (P61), CTV (P61), LIYV (P59), and LChV (P61). This protein is believed to be a putative heat shock 90 protein. As shown in Figure 9, two conserved motifs which were present in BYV (Agranovsky et al. "Nucleotide Sequence of the 3'-Terminal Half of Beet Yellow's Closterovirus RNA Genome Unique Arrangement of Eight Virus Genes," J. General Virology 72:15-24 (1991), which is hereby incorporated by reference) and CTV (Pappu et al., "Nucleotide Sequence and Organization of Eight 3' Open Reading Frames of the Citrus Tristeza Closterovirus Genome," Virology 199:35-46 (1994), which is hereby incorporated by reference) were also identified in the ORF4 of GLRaV-2.

30 ORF5 and ORF6 encode polypeptides with molecular mass of 24,803 Da and 21,661 Da, respectively. The start codon for both ORFs is in a favorable context for translation. ORF6 was identified as the coat protein gene of GLRaV-2 based on the sequence comparison with other closteroviruses. The calculated molecular mass of the protein product of ORF6 (21,662 Da) is in good agreement with the previously estimated 22-26 kDa based

on SDS-PAGE (Zimmermann et al., "Characterization and Serological Detection of Four
Closterovirus-like Particles Associated with Leafroll Disease on Grapevine," J.
Phytopathology 130:205-18 (1990); Boscia et al., "Nomenclature of Grapevine Leafroll-
Associated Putative Closteroviruses," Vitis 34:171-75 (1995), both of which are hereby
5 incorporated by reference).

Database searching with the deduced amino acid sequence of the ORF6 of
GLRaV-2 showed a similarity with the coat proteins of closteroviruses, BYV, BYSV, CTV,
LIYV, LChV, and GLRaV-3. At the nucleotide level, the highest percentage similarity was
with the coat protein of BYSV (34.8%); at the amino acid level, the highest percentage
10 similarity was with the coat proteins of BYV (32.7%) and BYSV (32.7%). As shown in
Figure 6A, alignment of the amino acid sequence of the coat protein and coat protein
duplicate of GLRaV-2 with respect to other closteroviruses revealed that the invariant amino
acid residues (N. R. G. D.) were present in both ORF5 and ORF6 of GLRaV-2. Two of
these amino acid residues (R and D) are believed to be involved in stabilization of molecules
15 by salt bridge formation and proper folding in the most conserved core region of coat proteins
of all filamentous plant viruses (Dolja et al., "Phylogeny of Capsid Proteins of Rod-Shaped
and Filamentous RNA Plant Viruses Two Families With Distinct Patterns of Sequence and
Probably Structure Conservation," Virology 184:79-86 (1991), which is hereby incorporated
by reference).

Identification of ORF6 as the coat protein gene was further confirmed by
Western blot following expression of a fusion protein, consisting of a 22 kDa of ORF6 CP
and a 42 kDa of maltose binding protein, produced by transformed *E. coli* as described in
Example 5 *supra*. As shown in Figure 6B, the putative phylogenetic tree of the coat protein
and coat protein duplicate of GLRaV-2 with those of other closteroviruses showed that
25 GLRaV-2 is more closely related to aphid transmissible closteroviruses (BYV, BYSV, and
CTV) (Candresse, "Closteroviruses and Clostero-like Elongated Plant Viruses," in
Encyclopedia of Virology, pp. 242-48, Webster and Granoff, eds., Academic Press, New
York (1994), which is hereby incorporated by reference) than to whitefly (LIYV) or
mealybug transmissible closteroviruses (LChV and GLRaV-3) (Raine et al., "Transmission
30 of the Agent Causing Little Cherry Disease by the Apple Mealybug *Phenacoccus aceris* and
the Dodder *Cuscuta Lupuliformis*," Canadian J. Plant Pathology 8:6-11 (1986); Jelkmann et
al., "Complete Genome Structure and Phylogenetic Analysis of Little Cherry Virus, a
Mealybug-Transmissible Closterovirus," J. General Virology 78:2067-71 (1997); Rosciglione
and Gugerli, "Transmission of Grapevine Leafroll Disease and an Associated Closterovirus

to Healthy Grapevine by the Mealybug *Planococcus ficus*," *Phytoparasitica* 17:63 (1989); Engelbrecht and Kasdorf, "Transmission of Grapevine Leafroll Disease and Associated Closteroviruses by the Vine Mealybug *planococcus-ficus*," *Phytophactica*, 22:341-46 (1990); Cabaleiro and Segura, 1997; Petersen and Charles, "Transmission of Grapevine

- 5 Leafroll-Associated Closteroviruses by *Pseudococcus longispinus* and *P. calceolariae*. *Plant Pathology* 46:509-15 (1997), all of which are hereby incorporated by reference).

ORF7 and ORF8 encode polypeptides of 162 amino acid with a molecular mass of 18,800 Da and of 206 amino acid with a molecular mass of 23,659 Da, respectively. Database searching with the ORF7 and ORF8 showed no significant similarity with any other
10 proteins. Nevertheless, these genes were of similar in size and location as those observed in the sequence of other closteroviruses, BYV (P20, P21), BYSV (P18, P22), and LChV (P21, P27) (Figure 7). However, conserved regions were not observed between the ORF7 or ORF8 and its counterparts in BYV, BYSV, and LChV.

- The 3' terminal untranslated region (3'-UTR) consists of 216 nucleotides.
15 Nucleotide sequence analysis revealed a long oligo(A) tract close to the end of the GLRaV-2 genome which is similar to that observed in the genome of BYV and BYSV (Agranovsky et al. "Nucleotide Sequence of the 3'-Terminal Half of Beet Yellow Closterovirus RNA Genome Unique Arrangement of Eight Virus Genes," *J. General Virology* 72:15-24 (1991); Karasev et al., "Organization of the 3'-Terminal Half of Beet Yellow Stunt Virus Genome
20 and Implications for the Evolution of Closteroviruses," *Virology* 221:199-207 (1996), both of which are hereby incorporated by reference). The genome of BYV ends in CCC, BYSV, and CTV ends in CC with an additional G or A in the double-stranded replicative form of BYSV (Karasev et al., "Organization of the 3'-Terminal Half of Beet Yellow Stunt Virus Genome and Implications for the Evolution of Closteroviruses," *Virology* 221:199-207 (1996), which
25 is hereby incorporated by reference) and CTV (Karasev et al., "Complete Sequence of the Citrus Tristeza Virus RNA Genome," *Virology* 208:511-20 (1995), which is hereby incorporated by reference), respectively. GLRaV-2 had CGC at the 3' terminus of the genome. Recently, a conserved 60 nt cis-element was identified in the 3'-UTR of three monopartite closteroviruses, which included a prominent conserved stem and loop structure
30 (Karasev et al., 1996). As shown in Figure 10, alignment of the 3'-UTR sequence of GLRaV-2 with the same regions of BYV, BYSV, and CTV showed the presence of the same conserved 60 nt stretch. Besides this cis-element, conserved sequences were not found in the 3' UTRs of GLRaV-2, BYV, BYSV, and CTV.

The closteroviruses studied so far (e.g., BYV, BYSV, CTV, LIYV, LChV, and GLRaV-3) have apparent similarities in genome organization, which include replication associated genes that consist of MT, HEL, and RdRP conserved domains and a five-gene array unique for closteroviruses (Dolja et al. "Molecular Biology and Evolution of Closteroviruses: Sophisticated Build-up of Large RNA Genomes," Annual Rev. Photopathology 32:261-85 (1994); Agranovsky "Principles of Molecular Organization, Expression, and Evolution of Closteroviruses: Over the Barriers," Adv. in Virus Res. 47:119-218 (1996); Jelkmann et al., "Complete Genome Structure and Phylogenetic Analysis of Little Cherry Virus, a Mealybug-Transmissible Closterovirus," J. General Virology 78:2067-71 (1997); Ling et al., "Nucleotide Sequence of the 3' Terminal Two-Thirds of the Grapevine Leafroll Associated Virus-3 Genome Reveals a Typical Monopartite Closterovirus," J. General Virology 79(5):1289-1301 (1998), all of which are hereby incorporated by reference).

The above data clearly shows that GLRaV-2 is a closterovirus. In the genome of GLRaV-2, two putative papain-like proteases were identified and an autoproteolytic cleavage process was predicted. The replication associated proteins consisting of MT, HEL, and RdRP conserved motifs were also identified, which were phylogenetically closely related to the replication associated proteins of other closteroviruses. A unique gene array including a small hydrophobic transmembrane protein, HSP70 homolog, HSP90 homolog, diverged CP and CP was also preserved in GLRaV-2. In addition, the calculated molecular mass (21,661 Da) of the coat protein (ORF6) of GLRaV-2 is in good agreement with that of the other closteroviruses (22 to 28 kDa) (Martelli and Bar-Joseph, "Closteroviruses: Classification and Nomenclature of Viruses," Fifth Report of the International Committee on Taxonomy of Viruses, Francki et al., eds., Springer-Verlag Wein, New York, p. 345-47 (1991); Candresse and Martelli, "Genus *Closterovirus*," in Virus Taxonomy. Report of the International Committee on Taxonomy of Viruses, Murphy et al., eds., Springer-Verlag., NY, p. 461-63 (1995), both of which are hereby incorporated by reference). Two ORFs downstream of the CP are of similar, in size and location, to those observed in the genome of BYV. Furthermore, lack of a poly(A) tail at the 3' end of GLRaV-2 is also in good agreement with other closteroviruses. Like all other closteroviruses, the expression of ORF1b is suspected to occur via a +1 ribosomal frameshift and the 3'proximal ORFs are probably expressed via formation of a nested set of subgenomic RNAs. Since the slippery sequence, stem-loop and pseudoknot structure involved in the frameshift of BYV were absent in GLRaV-2, the +1 frameshift of GLRaV-2 might be the same as proposed for CTV (Karasev et al., "Complete

Sequence of the Citrus Tristeza Virus RNA Genome," Virology 208:511-20 (1995), which is hereby incorporated by reference) and BYSV (Karasev et al., "Organization of the 3'-Terminal Half of Beet Yellow Stunt Virus Genome and Implications for the Evolution of Closteroviruses," Virology 221:199-207 (1996), which is hereby incorporated by reference).

5 Overall, GLRaV-2 is more closely related to monopartite closteroviruses BYV, BYSV, and CTV than to GLRaV-3 (Figure 7) (Ling et al., "Nucleotide Sequence of the 3' Terminal Two-Thirds of the Grapevine Leafroll Associated Virus-3 Genome Reveals a Typical Monopartite Closterovirus," J. General Virology 79(5):1289-1301 (1998), which is hereby incorporated by reference), even though the latter causes similar leafroll symptoms in
10 grapevine (Rosciglione and Gugerli, "Maladies de l'Enroulement et du Bois Strie de la Vigne: Analyse Microscopique et Serologique (Leafroll and Stem Pitting of Grapevine: Microscopical and Serological Analysis)," Rev Suisse Viticult Arboricult Horticulture 18:207-11 (1986); Hu et al., "Characterization of Closterovirus-Like Particles Associated with Grapevine Leafroll Disease," J. Phytopathology 128:1-14 (1990), both of which are
15 hereby incorporated by reference).

Closteroviruses are a diverse group with complex and heterogeneous genome organizations. So far, GLRaV-2 is the only closterovirus that matches with the genome organization of BYV, the type member of the genus *Closterovirus*. In addition, the genomic RNA of GLRaV-2 is about the same size as that of BYV; however, the transmission vector of
20 GLRaV-2 is unknown. The genome organization of GLRaV-2 is more closely related to the aphid transmissible closteroviruses (BYV and CTV) than to whitefly (LIYV) or mealybug transmissible closteroviruses (LChV and GLRaV-3). Thus, it is possible that GLRaV-2 is transmitted by aphids. Aphid transmission experiments with GLRaV-2 should provide information that might help develop methods for further control of GLRaV-2.

25 A total of 15,500 nucleotides or over 95% of the estimated GLRaV-2 genome has been cloned and sequenced. GLRaV-2 and GLRaV-3 (Ling et al., "Nucleotide Sequence of the 3' Terminal Two-Thirds of the Grapevine Leafroll Associated Virus-3 Genome Reveals a Typical Monopartite Closterovirus," J. General Virology 79(5):1289-1301 (1998), which is hereby incorporated by reference) are the first grapevine leafroll associated
30 closteroviruses that have been almost completely sequenced. The above data clearly justify the inclusion of GLRaV-2 into the genus *Closterovirus*. In addition, the information regarding the genome of GLRaV-2 would provide a better understanding of this and related GLRaVs, and add fundamental knowledge to the group of closteroviruses.

Example 7 - Construction of the CP Gene of GLRaV-2 in Plant Expression Vector

GLRaV-2 infected Vitis vinifera, cv Pinot Noir grapevines originated from a vineyard in central New York was used as the virus isolate, from which the cp gene of GLRaV-2 was identified. Based on the sequence information, two oligonucleotide primers have been designed. The sense primer CP-96F (SEQ. ID. No. 21) starts from the ATG initiation codon of the coat protein gene and the complementary primer CP-96R (SEQ. ID. No. 22) starts from 56 nucleotides downstream of the stop codon of the CP gene. A Nco I restriction site (11 bp in SEQ. ID. No. 21 and 13 bp in SEQ. ID. No. 22) is introduced in the beginning of both primers to facilitate the cloning. The coat protein gene of GLRaV-2 was amplified from dsRNA extracted from GLRaV-2 infected grapevine using reverse transcriptase polymerase chain reaction (RT-PCR). The PCR-amplified CP product was purified from low melting temperature agarose gel, digested with Nco I and cloned into the same enzyme digested plant expression vector pEPT8 (shown at Figure 11). After screening, the orientation of recombinant construct was checked by using the internal restriction site of the CP gene and directly sequencing the CP gene. The recombinant construct with translatable (sense) full length coat protein gene, pEPT8CP-GLRaV2, was going through for the further cloning. The plant expression cassette, which consisted of a double cauliflower mosaic virus (CaMV) 35S-enhancer, a CaMV 35S-promoter, an alfalfa mosaic virus (ALMV) RNA4 5' leader sequence, a coat protein gene of GLRaV-2 (CP-GLRaV-2), and a CaMV 35S 3' untranslated region as a terminator, was cut using the EcoR I restriction enzyme, isolated from low melting point temperature agarose gel, and cloned into the same restriction enzyme treated binary vector pGA482GG or pGA482G (a derivative of pGA482 (An et al., "Binary Vectors," in Plant Molecular Biology Manual, pp. A3:1-19, Gelvin and Schilperroot, eds., Kinwer Academic Publishers, Dordrecht, Netherlands (1988), which is hereby incorporated by reference). The resulting recombinants constructs are pGA482GG/EPT8CP-GLRaV2 (shown at Figure 11A), which contain both neomycin phosphotransferase (npt II) and β -glucuronidase (GUS) at the internal region of the T-DNA, and pGA482G/EPT8CP-GLRaV2 (shown at Figure 11B) without GUS. These recombinants constructs were separately introduced by electroporation into disarmed avirulent *Agrobacterium tumefaciens* strain C58Z707. The *Agrobacterium tumefaciens* containing the vector was used to infect *Nicotiana benthamiana* wounded leaf disks according to the procedure essentially described by Horsch et al., "A Simple and General Method for

Transferring Genes into Plants," Science 277:1229-1231 (1985), which is incorporated herein by reference.

Example 8 - Analysis of Transgenic *Nicotiana benthamiana* Plants with the CP Gene of GLRaV-2

NPT II-ELISA: Double-antibody sandwich enzyme linked immuosorbent assay (DAS-ELISA) was used to detect the npt II enzyme with an NPT II-ELISA kit (5' prime to 3' prime, Inc., Boulder, Co.).

Indirect ELISA: Polyclonal antibodies to GLRaV-2, which were prepared from the coat protein expressed in *E. coli*, were used. Plates were coated with homogenized samples in extraction buffer (1:10, w/v) (phosphate buffered saline containing 0.05% Tween 20 and 2% polyvinyl pyrrolidone) and incubated overnight at 4°C. After washing with phosphate buffered saline containing 0.05% Tween 20 (PBST), the plates were blocked with blocking buffer (phosphate buffered saline containing 2% BSA) and incubated at room temperature for 1 hr. The anti-GLRaV-2 IgG was added at 2 µg/ml after washing with PBST. After incubation at 30 C for 4 hr, the plates were washed with PBST, and the goat anti-rabbit IgG conjugate of alkaline phosphatase (Sigma) was added at 1:10,000 dilution. The absorbance was measured at 405 nm with a MicroELISA AutoReader. In addition, Western blot was also performed according to the method described by Hu et al., "Characterization of Closterovirus-like Particle Associated Grapevine Leafroll Disease," J. Phytopathology 128:1-14, (1990), which is incorporated herein by reference.

PCR analysis: Genomic DNA was extracted from leaves of putative transgenic and non-transgenic plants according to the method described by Cheung et al., "A Simple and Rapid DNA Microextraction Method for Plants, Animal, and Insect Suitable for RAPD and other PCR analysis," PCR Methods and Applications 3:69 (1996), which is incorporated herein by reference. The extracted total DNA served as the template for PCR reaction. The primers CP-96F and CP-96R (SEQ. ID. Nos. 21 and 22, respectively) for the CP gene of GLRaV-2, as well as npt II 5'- and 3'- primers were used for PCR analysis. PCR reaction was performed at the 94°C x 3 min for one cycle, followed by 30 cycles of 94° C x 1 min, 50° C x 1 min, and 72° C x 2:30 min with an additional extension at 72° C for 10 min. The PCR product was analyzed on agarose gel.

After transformation, a total of 42 kanamycin resistant *Nicotiana benthamiana* lines (R₀) were obtained, of which the leaf samples were tested by NPT II enzyme activity.

Among them, 37 lines were NPT II positive by ELISA, which took about 88.0% of total transformants. However, some of NPT II negative plants were obtained among these selected kanamycin resistant plants. All of the transgenic plants were self-pollinated in a greenhouse, and the seeds from these transgenic lines were germinated for further analysis.

5 The production of GLRaV-2 CP in transgenic plants was detected by indirect ELISA prior to inoculation, and the results showed that GLRaV-2 CP gene expression was not detectable in all transgenic plants tested. This result was further confirmed with Western blot. Using the antibody to GLRaV-2, the production of the CP was not detected in the transgenic and nontransgenic control plants. However, a protein of expected size (~22 kDa)
10 was detected in GLRaV-2 infected positive control plants. This result was consistent with the ELISA result. The presence of the CP gene of GLRaV-2 in transgenic plants was detected from total genomic DNA extracted from plants tissue by PCR analysis (Figure 12). The DNA product of expected size (653 bp) was amplified from twenty tested transgenic lines, but not in non-transgenic plants. The result indicated that the CP gene of GLRaV-2 was present at
15 these transgenic lines, which was also confirmed by Northern blot analysis.

Example 9 - R₁ and R₂ transgenic *Nicotiana benthamiana* Plants Are Resistant to GLRaV-2

20 Inoculation of transgenic plants: GLRaV-2 isolate 94/970, which was originally identified and transmitted from grapevine to *Nicotiana benthamiana* in South Africa (Goszczynski et al., "Detection of Two Strains of Grapevine Leafroll-Associated Virus 2," *Vitis* 35:133-35 (1996), which is incorporated herein by reference), was used as inoculum. The CP gene of isolate 94/970 was sequenced; and it is identical to the CP gene
25 used in construction. *Nicotiana benthamiana* is an experimental host of GLRaV-2. The infection on it produces chlorotic and occasional necrotic lesions followed by systemic vein clearing. The vein clearing results in vein necrosis. Eventually the infected plants died, starting from the top to the bottom.

At five to seven leaf stage, two youngest apical leaves were challenged with
30 GLRaV-2 isolate 94/970. Inoculum was prepared by grinding 1.0 g GLRaV-2 infected *Nicotiana benthamiana* leaf tissue in 5 ml of phosphate buffer (0.01M K₂HPO₄, PH7.0). The tested plants were dusted with carborundum and rubbed with the prepared inoculum. Non-transformed plants were simultaneously inoculated as above. The plants were observed for symptom development every other day for 60 days after inoculation. Resistant R₁ transgenic

plants were carried on to R2 generation for further evaluation.

Transgenic progenies from 20 R₀ lines were initially screened for the resistance to GLRaV-2 followed by inoculation with GLRaV-2 isolate 94/970. The seedlings of the transgenic plants (NPT II positive), and nontransformed control plants were inoculated with GLRaV-2. After inoculation, the reaction of tested plants were divided into three types: highly susceptible (i.e. typical symptoms were observed two to four weeks postinoculation); tolerant (i.e. no symptom was developed in the early stage and typical symptoms was shown four to eight weeks postinoculation); and resistant (i.e. the plants remained asymptomatic eight weeks postinoculation). Based on the plant reaction, the resistant plants were obtained from fourteen different lines (listed in Table 1 below). In each of these fourteen lines, there was no virus detected within these plants by ELISA at 6 weeks postinoculation. In contrast, GLRaV-2 was detected in symptomatic plants by indirect ELISA. In the other six lines, although there were a few plants with some kind of delay in symptom development, all the inoculated transgenic plants died at three to eight weeks postinoculation. Based on the initial screening results, five representative lines consisting of three resistant lines (1, 4, and 19) and two susceptible lines (12 and 13) were selected for the further analysis.

Table 1

No. Line	No.	Reaction of Tested Plants		
		HS	T	HR
line 1	39	14	3	22
line 2	36	7	6	23
line 3	38	11	4	23
line 4	31	4	5	22
line 5	33	6	13	14
line 6	36	4	16	16
line 7	32	5	9	18
line 8	37	22	9	6
line 9	36	9	12	15
line 10	14	13	1	0
line 11	13	11	2	0
line 12	17	16	1	0
line 13	16	14	0	0
line 14	17	17	0	0
line 15	32	30	2	0
line 16	33	6	13	14
line 17	12	0	1	11
line 19	15	0	0	15
line 20	19	3	0	16
line 21	14	1	3	10
control	15	15	0	0

Table 1

		Reaction of Tested Plants		
No. Line	No.	HS	T	HR
No Line: include transgenic lines and nontransformed control; No: the number of transgenic and nontransformed plants; HS: highly susceptible, typical symptoms were observed two to four weeks after inoculation; T: tolerant, the symptoms were observed five to eight weeks after inoculation; and HR: plants remain without asymptomatic after eight weeks inoculation.				

Table 2 below shows the symptom development in transgenic plants relative to non-transgenic control plants in the five selected lines in separate experiments. Non-transgenic control plants were all infected two to four weeks after inoculation, which showed typical GLRaV-2 symptoms on *Nicotiana benthamiana*, including chlorotic and local lesions followed by systemic vein clearing and vein necrosis on the leaves. Three of the tested lines (1, 4, and 19) showed some resistance that was manifested by either an absence or a delay in symptom development. Two other lines, 12 and 13, developed symptoms at nearly the same time as the non-transformed control plants. From top to bottom, the leaves of infected plants gradually became yellow, wilted, and dried, and, eventually, the whole plants died. No matter when infection occurred, the eventual result was the same. Six weeks after inoculation, all non-transgenic plants and the susceptible plants were dead. Some tolerant plants started to die. In contrast, the asymptomatic plants were flowering normally and pollinating as the non-inoculated healthy control plants (Figure 13).

Table 2

No. Line	No.	Reaction of Tested Plants		
		HS	T	HR
line 1	19	5	6	8
line 4	15	9	1	5
line 12	16	14	2	0
line 13	18	13	5	0
line 19	13	10	0	3
non-transgenic	24	23	1	0
No. Line: include transgenic lines and nontransformed control; No.: Number of transgenic and nontransformed plants tested; HS: highly susceptible; typical symptoms were observed two to four weeks after inoculation; T: tolerant, the symptoms were observed five to eight weeks postinoculation; and HR: plants remain without asymptomatic after eight weeks inoculation.				

ELISA was performed at 6 weeks postinoculation to test the GLRaV-2 replication in the plants. Presumably, the increased level of CP reflected virus replication. The result showed that the absorbance value in symptomatic plants reached (OD) 0.7 to 3.2,

compared to (OD) 0.10-0.13 prior to inoculation. In contrast, GLRaV-2 was not detected in asymptomatic plants, of which the absorbance value was the same or nearly the same as that of healthy nontransformed control plants. The data confirmed that virus replicated in symptomatic plants, but not in asymptomatic plants. The replication of GLRaV-2 was suppressed in asymptomatic plants. This result implicated that another mechanism other than the CP-mediated resistance was probably involved.

Three R2 progenies derived from transgenic resistant plants of lines 1, 4, and 19 were generated and utilized to examine the stable transmission and whether resistance was maintained in R2 generation. These results are shown in Table 3 below. NPT II analysis revealed that R2 progeny were still segregating. The CP expression in R2 progeny was still undetectable. After inoculation, all the nontransgenic plants were infected and showed GLRaV-2 symptoms on the leaves after 24 days postinoculation. In contrast, the inoculated transgenic R₂ progeny showed different levels of resistance from those highly susceptible to highly resistant. The tolerant and resistant plants were manifested by a delay in symptom development and absence of symptoms, respectively. At 6 weeks postinoculation, GLRaV-2 was detected in the tolerant symptomatic infected plants by indirect ELISA; but not in asymptomatic plants. This result indicated that virus replication was suppressed in these resistant plants, which was confirmed by Western blot. These resistant plants remained asymptomatic eight weeks postinoculation, and they were flowering normally and pollinating.

Table 3

No. Line	No. Plants	NPT II positive/negative	HS	Reaction of Tested Plants	
				T	HR
line 1/22	12	12/20	3	3	6
line 1/30	11	8/3	7	2	2
line 1/31	11	10/1	6	3	2
line 1/35	10	10/0	4	6	0
line 1/41	8	7/1	2	2	4
line 4/139	12	11/1	4	4	3
line 4/149	10	7/3	4	5	1
line 4/152	10	8/2	9	0	1
line 4/174	9	8/1	4	0	4
line 19/650	11	10/1	7	0	2
line 19/657	12	12/0	6	2	4
line 19/659	12	8/4	5	2	5
line 19/660	10	8/2	3	6	1
non-transformed	12	0/12	12	0	0

CK

HS: highly susceptible, typical symptoms were observed two to four weeks after inoculation;

T: tolerant, the symptoms were observed five to eight weeks postinoculation; and

HR: plants remain asymptomatic at eight weeks postinoculation.

Example 10 - Evidence for RNA-Mediated Protection in Transgenic Plants

Northern blot analysis: Total RNA was extracted from leaves prior to inoculation following the method described by Napoli et al., Plant Cell 2:279-89 (1990), which is hereby incorporated by reference. The concentration of the extracted RNA was measured by spectrophotometer at OD 260. About 10 g of total RNA was used for each sample. The probe used was the 3' one third of GLRaV-2 CP gene, which was randomly labeled with ³²P (α -dATP) using Klenow fragment of DNA polymerase I.

Using a DNA corresponding to the 3' one third CP gene sequence as probe, a single band was detected in the RNA extracted from susceptible plants from R1 progeny of lines 5, 12, and 13 by Northern hybridization. There was little or no signal detected in the transgenic plants from R1 progeny of line 1, 4, and 19. This RNA is not present in nontransformed control plants. The size of the hybridization signal was estimated to an approximately 0.9 kb nucleic acid, which was about the same as estimated (Figure 14). In lines of 1, 4, and 19, the steady state level of RNA expression was also low in R2 progeny. This data showed that susceptible plants from lines 12 and 13 had high mRNA level and all transgenic plants from lines 1, 4, and 19 had low mRNA level.

Example 11 - Transformation and Analysis of Transgenic Grapevines with the CP Gene of GLRaV-2

Plant materials: The rootstock cultivars Couderc 3309 (3309C) (*V. riparia* x *V. rupestris*), *Vitis riparia* 'Gloire de Montpellier' (Gloire), Teleki 5C (5C) (*V. berlandieri* x *V. riparia*), Millardet et De Grasset 101-14 (101-14 MGT) (*V. riparia* x *V. rupestris*), and Richter 110 (110R) (*V. rupestris* x *V. berlandieri*) were utilized. Initial embryogenic calli of Gloire were provided by Mozsar and Süle (Plant Protection Institute, Hungarian Academy of Science, Budapest). All other plant materials came from a vineyard at the New York State Agricultural Experiment Station, Geneva, NY. Buds were removed from the clusters and surface sterilized in 70% ethanol for 1-2 min. The buds (from the greenhouse and the field) were transferred to 1% sodium hypochlorite for 15 min, then rinsed three times in sterile, double-distilled water. Anthers were excised aseptically from flower buds with the aid of a stereo microscope. The pollen was crushed on a microscope slide under a coverslip with a drop of acetocarmine to observe the cytological stage. This was done to determine which stage was most favorable for callus induction.

Somatic embryogenesis and regeneration: Anthers were plated under aseptic conditions at a density of 40 to 50 per 9 cm diameter Petri dish containing MSE. Plates were cultured at 28°C in the dark. Callus was initiated, and, after 60 days, embryos were induced and were transferred to hormone-free HMG medium for differentiation. Torpedo stage embryos were then transferred from HMG to MGC medium to promote embryo germination. Cultures were maintained in the dark at 26-28°C and transferred to fresh medium at 3-4 week intervals. Elongated embryos were transferred to rooting medium in baby food jars (5-8 embryos per jar). The embryos were grown in a tissue culture room at 25°C with a daily 16 h photoperiod (76 :mol. s) to induce shoot and root formation. After plants developed roots, they were transplanted to soil in the greenhouse.

Transformation: The protocols used for transformation were modified from those described by Scorza et.al., "Transformation of Grape (*Vitis vinifera* L.) Zygotic-derived Somatic Embryos and Regeneration of Transgenic Plants," *Plant Cell Rpt.* 14:589-92 (1995), which is hereby incorporated by reference. Overnight cultures of *Agrobacterium* strain C58Z707 or LBA4404 were grown in LB medium at 28°C in a shaking incubator. Bacteria were centrifuged for 5 min at 3000-5000 rpm and resuspended in MS liquid medium (OD 1.0 at A600 nm). Calli with embryos were immersed in the bacterial suspension for 15-30 min, blotted dry, and transferred to HMG medium with or without acetosyringone (100 µM). Embryogenic calli were co-cultivated with the bacteria for 48 h in the dark at 28°C. Then, the plant material was washed in MS liquid plus cefotaxime (300 mg/ml) and carbenicillin (200 mg/ml) 2-3 times. To select transgenic embryos, the material was transferred to HMG medium containing either 20 or 40 mg/L kanamycin, 300 mg/L cefotaxime, and 200 mg/L carbenicillin. Alternatively, after co-cultivation, embryogenic calli were transferred to initiation MSE medium containing 25 mg/l kanamycin plus the same antibiotics listed above. All plant materials were incubated in continuous dark at 28°C. After growth on selection medium for 3 months, embryos were transferred to HMG or MGC without kanamycin to promote elongation of embryos. They were then transferred to rooting medium without antibiotics. Nontransformed calli were grown on the same media with and without kanamycin to verify the efficiency of the kanamycin selection process.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

We claim:

1. An isolated RNA molecule encoding protein or polypeptide of a grapevine leafroll virus (type 2).

2. The isolated RNA molecule according to claim 1, wherein the protein or polypeptide is selected from a group consisting of a polyprotein, an RNA-dependent RNA polymerase, a heat shock 70 protein, a heat shock 90 protein, a diverged coat protein, and a coat protein.

3. An isolated DNA molecule encoding a protein or polypeptide of a grapevine leafroll virus (type 2).

4. The isolated DNA molecule according to claim 3, wherein the protein or polypeptide is selected from a group consisting of a polyprotein, an RNA-dependent RNA polymerase, a heat shock 70 protein, a heat shock 90 protein, a diverged coat protein, and a coat protein.

5. An expression system comprising a DNA molecule according to claim 3 in a vector heterologous to the DNA molecule.

6. The expression system according to claim 5, wherein the protein or polypeptide is selected from a group consisting of a polyprotein, an RNA-dependent RNA polymerase, a heat shock 70 protein, a heat shock 90 protein, a diverged coat protein, and a coat protein.

7. A host cell transformed with a heterologous DNA molecule according to claim 3.

8. The host cell according to claim 7, wherein the host cell is selected from the group consisting of *Agrobacterium vitis* and *Agrobacterium tumefaciens*.

9. The host cell according to claim 7, wherein the host cell is selected from a group consisting of a grape cell, a citrus cell, a beet cell, and a tobacco cell.

10. The host cell according to claim 7, wherein the protein or polypeptide is selected from a group consisting of a polyprotein, an RNA-dependent RNA-polymerase, a heat shock 70 protein, a heat shock 90 protein, a diverged coat protein, and a coat protein.

11. A transgenic plant cultivar comprising the DNA molecule according to claim 3.

12. The transgenic plant cultivar according to claim 11, wherein the plant cultivar is selected from a group consisting of a grape plant cultivar, a citrus plant cultivar, a beet plant cultivar, and a tobacco plant cultivar.

13. The transgenic plant cultivar according to claim 11, wherein the protein or polypeptide is selected from a group consisting of a polyprotein, an RNA-dependent RNA polymerase, a heat shock 70 protein, a heat shock 90 protein, a diverged coat protein, and a coat protein.

14. A method of imparting grapevine leafroll virus resistance to a *Vitis* scion or rootstock cultivar or a *Nicotiana* cultivar comprising the steps of:

(a) transforming of cells of a *Vitis* scion or rootstock cultivar or cells of a *Nicotiana* cultivar with a DNA molecule encoding a protein or polypeptide of a grapevine leafroll virus (type 2) according to claim 3; and

(b) regenerating a *Vitis* scion or rootstock cultivar or a *Nicotiana* cultivar from said transformed cells.

15. The method according to claim 14, wherein the protein or polypeptide is selected from a group consisting of a polyprotein, an RNA-dependent RNA polymerase, a heat shock 70 protein, a heat shock 90 protein, and a coat protein.

16. The method according to claim 14, wherein the grapevine leafroll virus GLRaV-2.

17. The method according to claim 14, wherein said transforming is *Agrobacterium* mediated.

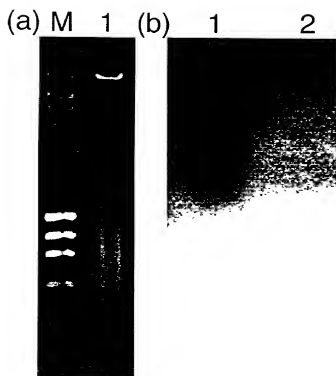
18. The method according to claim 14, wherein said transforming comprises: propelling particles at grape or tobacco plant cells under conditions effective for the particles to penetrate into the cell interior and introducing an expression vector comprising the DNA molecule into the cell interior.

ABSTRACT OF THE DISCLOSURE

The present invention relates to isolated proteins or polypeptides of grapevine leafroll virus (type 2). The encoding DNA molecules either alone in isolated form or in an expression system, a host cell, or a transgenic grape plant are also disclosed. Other aspects of the present invention relates to a method of imparting grapevine leafroll resistance to grape and tobacco plants by transforming them with the DNA molecules of the present invention, a method of imparting beet yellows virus resistance to a beet plant, a method of imparting tristeza virus resistance to a citrus plant, and a method of detecting the presence of a grapevine leafroll virus, such as GRLaV-2, in a sample.

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00513446.07100



FIGURES 1A, 1B

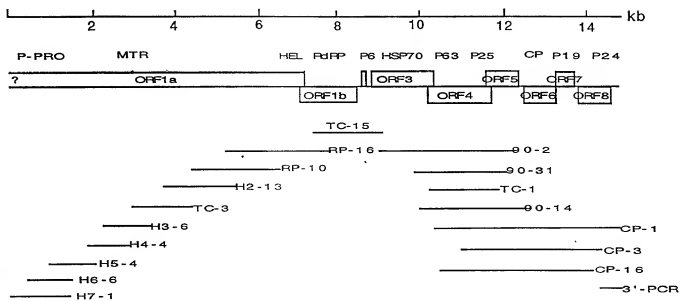


FIGURE 2

a

GLRaV2-PRO₁ SRVIYPDGRGCLAHMRYLCAPYCRPPRESFDYALGMPTVARLRACVEKNFVGEACGIALRGYYTSRRNVHCDYDSAXVKYPRNLSSGRIG/G
 GLRaV2-PRO₂ TRIRYPNGFCYLAHCRVYACFLRGRFPKRFDIGAPFTAALKLRNRMVSELGERSLGLNLYGAYTSRGRVPHCDYDAKFIKDLRLMSAVIA/G
 BYV-P-PRO LQYRPGBGCLYLAAALCCALQKRTFREDDFVGMYPYTKFVFAKRLTEKLGPSALKHPVGRQVSRSLFHCYDVASAFSPFYSYLPRIIG/G
 ConsensusG.CYLAH.....CA...R.F.....G..PT.....G.....G...SR...HCD.....I./G

b

GLRaV2-MTR MSEATQNSLTRYFPQFELKFSSHSSDHPAAAAASRLLENETLWRLCGNSVSDIGGCCPLFLHLSKTKQRRVHVCPVLDGKDAQRRVVRDLQ
 BYV-MTR MGEAVQSGLTRAYPQFNLSPTHSVSDHPAAAGSRLLENETLWRLCGNSVSDIGGCCPLFLHLSKTKQRRVHVCPVLDGKDAQRRVVRDLQ
 GLRaV2-MTR YSNVRLG-DDDKILEGRPNIDICHYPLGACDHESSAMMMQVVDASLYETICGAMIKKSRITYLTMVTPGFEFLDGRCEVYMESLDCEIEV
 BYV-MTR ARGVLNENLSREQLEVAQARVSVCPHTLGCNCKNSDVLIMVQVYDASLNETASAMVLKESKVAYLTMVTPGELLDEREAFADALGDCVVV
 GLRaV2-MTR DVHADVMYKFGSSCYSHKLSIITDKIMTTPYTLTGGFLPSVEMYEVRMGVNYFKITKSEVSPSISCTKLLRYRRANSDDVVKVLPFRD
 BYV-MTR DTRDMVQYKFGSSCYCHKLSNIKLSIMLPAPFTFSGNLFPSVEMYENRMGVNYFKITRSAYSPEIRGVKTLRYRRACTEVVQVKLPFRD

c

GLRaV2-Hel1 FVFTNSSVDILLYEAPGGGKTTLLIDSFLVKFKGGEVSMILTANKSSQVEILKKEVEVSMIECQKKRKRSPKKSIIYTDAYLMHHR
 BYV-Hel1 FFTTNLSANVLLYEAPGGGKTTLLIKVCFETFSK---VNSLILTANKSSREELAKVNRIVLD-EGDTPLQTRDR---ILTIDSYLMNNR
 GLRaV2-Hel1 GCDADVLFIDECVMHAGSVLACIEFTRCHKVMIFGDSRQIHYIERNELDKCLYGLDRFVDLQCRVYGNISYRCPPWDVCWALSTVYGNL
 BYV-Hel1 GLTKCVLYLDECFVMHAGAAVACIEFTKCDASAILFGDSRQIRYGRCSBELTAVLSDLNRFVDDDSRVYGEVSRCPWDVCWALSTFYFKT
 GLRaV2-Hel1 IATVKGESEKSSMRINEINSVDOLVPDVGSTFLCMLQSEKLEISKH---IRKGLTKIANLVVHEAQGETYARNVLRKLPQDEPPKS
 BYV-Hel1 VATTNLVSAAQSSMQVREIESVDDVEYSSEFVYTLMLQSEKDLKLSFGKRSRSVEKPTVLTVLHAEAQGETYARKVNLVVRTKFQDEDDPFS
 GLRaV2-Hel1 IRHITVALSRHTDSLTYNVLAARRGDATCDAIQKAAELVNKFRVFPFSPGGS
 BYV-Hel1 ENHITVALSRHVESLTVSVLSSKRRDIAQAIVKAKQLVDAYRVVFPFSPGGS

d

GLRaV2-RdRP ICRFLMVKRDAKVLSDSCLTKHSAQNMIFHRKSNIAIFSP1FNEVKNRIMCCLKPNIKFFTEMTNRDFAVSVSNMGLDDDDVYHIGEV
 BYV-RdRP ITTFKLMVKRDAKVLSDSCLVKHPPAQNIMFHRKAVNAIFSP2CFDEKRNVTCTNSNIVFTEMTNSTLASIAKEMLGSEHVYVNGEII
 GLRaV2-RdRP DFKSKYDQSDAFVKAPEEVMYKELGVDELLAIWMCGERLSIANPLDGLQSFTEIENQRKSGASNTWIGNSLVTLGILSLYIVDNRFEALY
 BYV-RdRP DFKSKYDQSDAFIKSFERTLYSAGFDDELLDVMQGEYSNATPLDGLQSFSDVNRKSGASNTWIGNSIETLILGILSMFYNTNRKFLAF
 GLRaV2-RdRP ISGDDSLIFSRSEISNIYADDICTDMGFEETKPMSPSVYFCSKFVVMCGHKTFFVDPDKYLKVLKAVKEDVSMDFLFTETFSFKDLTSDF
 BYV-RdRP VSGDDSLIFSESPIRNSADAMCTELGPFETKFLTPSVYFCSKFVVMTHGDFVDPDKYLKVLKAVKEDVDEDFLFEVFTSPFRDLTKDL
 GLRaV2-RdRP NDERLIQKLAELVALKYEVQTNPTTIAL
 BYV-RdRP VDERVIELLTLVHSKYGYESGDTYAL

FIGURES 3A, 3B, 3C, 3D

a

GLRaV-2	CAUGAUAGCCAGCGGUGUU <u>UAGG</u> GUGAGUUGGUGCGAGGCGAUUCCGGUGA
EW	CACGACCCGCGAGCGGGUU <u>UAGC</u> TGAGUUGCGUUGCGAGGCGAUUCCUAGAGG
EVSV	CACGAUGAUCAGCGCGUU <u>UAGG</u> GUGAGUUGGUGCGAGGCGAUUCCUAAAGG
CIV	CACGAACCGCGGUGGUGUGGUGAGUAGGUCACAGCAUUCUCCAGGA
Consensus	CA,GA.....CG,GUU..GC....U....UC,CA,GC,AU,CC....AG.

b

GLRaV-2	H D K Q R V <u>S</u> V V R S Q A I P R R
EW	H D P Q R V <u>S</u> S I R S Q A I P K R
EVSV	H D E Q R V <u>S</u> V V R S Q A I P K R
CIV	H E P A R V <u>G</u> V V R S Q A I P P R
Consensus	H . . . R V . . . R S Q A I P . R

FIGURES 4A, 4B

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A
 GLRaV2-HSP70 M V P G L D F G T I F S T V C Y K D G R V F S F K Q N N S A Y I P T Y L Y L F S D S N H M T G Y E A S L M S N L K V K G S F Y R D L K R W G C D S S N L D A V L D R L K P
 BV-HSP70 M V P G L L D F G T I F S S V C A Y V G E E L Y L F K Q R D S A Y I P T V F L H S D T Q E V A P G V D A E V L S N D L S V R G G F Y R D L K R W I G C D E E N Y R D Y L E K L K P

B
 GLRaV2-HSP70 H Y S V R L V K I G S G L N E T V S I G N F G G T V K S E A H L P G L I A L F T K A V I S C A B A F A C T I C T G V I C S V P A N Y D S V Q R N F T D Q C V S L S G Y Q C V M M N
 BV-HSP70 H Y K T E L L K V A Q S S K S T V K L D C Y S G T V P Q N A L P G L I A T F V K A L I S T A S E A F K Q C T G V I C S V P A N Y A N C L Q R S P T E S C V N L S G Y P C V M M N

C D
 GLRaV2-HSP70 E P S A A A L S A C N S I G K S A N L A V Y D F G G G T F D V S I I S Y R N I F V W R A S G G D N L G R V D R A F L T H L F S L T S L E P D L T L D T S N L K E S L S K T
 BV-HSP70 E P S A A A L S A C S R I K G A T S P V L W Y D F G G G T F D V S I S A L N I F V W R A S G G D N L G R D I I K A F V E H L Y N K A Q L P V N Y K C I D T S P L K E S L S K K

E
 GLRaV2-HSP70 D A E I V Y T L G V D G R K E D V R V N K N I L T S V M L P Y V N R I L K I L E S T L K S A K S M N E S A R V K C D L V L I G G S S Y L P G L A D V L T K H Q S V D R I L R V S
 BV-HSP70 V S P I N F P V S B Q G R V D V L V N S E L A B A A P F V E R T I K I V K E Y E K Y C S S M R L E N V K A L I M V G G S S Y L P G L L S R L S S I P F V D E C I V L P

F G
 GLRaV2-HSP70 D P R A A V A G C A L Y S S C L S G S G G L L I D C A A H I V A I A D R S C H Q I C A P A G A P I P F S G S M P L Y L A R V N K N S O R E V A V F E G E V K C P K N R K I C
 BV-HSP70 D A R A A V A G C A L Y S A C L R N D S E M L L V D C A A H N I S T S S K Y C B S I V C P A G S P I P T G V R I V N M T G S N A V Y S A A L F E G E V K C R L N K R I F

H
 GLRaV2-HSP70 G A N I R F P D I G V T G S Y A P V I F Y M D F S I S S V G A V S F V R G P E G K Q V S L T G T P A Y N F S S V A L G S R S V R E L H I S L N N K V F L G L L H R K A D R R I
 BV-HSP70 P G D V L G N G V T G S A T R I V P L T L E I N V S S V G T I S F S L A G P T G V K L I G N N A Y D F S S Y Q L G R V A D L H G H N S D K V K L I H A L T Y Q P F O R K

GLRaV2-HSP70 L P I K D E A I R Y A D G I -- D I A D V L K E Y K S Y A A S A L P D E D V E L L I G S V Q V K L R G S R L E E I P L .
 BV-HSP70 K L I D G K A L F L K R L T A D Y R F R A R K P S S Y D A V L -- N S S E L L I G R I T P K I L R G S R V E K I D - V

FIGURE 5

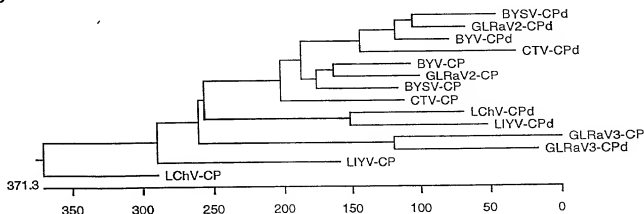
a

GLRaV2-CP M-----ELMSDN-----L-----SNLVTID-----ASSINGVKKLLSAEVRKMLVQK--GAPNE
 GLRaV2-CPd M--SSNTSPVAGGLEALETSSWLTIR--KEAVDRF-----PNEIKNNYSVDSRLSDSEVKEVLEKSEKSF--SELASTDE
 BYV-CP MESAEP-----ISA-----IATFENSLAD-----QTCLHGECCKLRKNEFEECLTK--GVPEd
 BYV-CPd MIAFEARGDLTH-----PIFENIRDAEITF-----PNSYDLAEYSVANRNNKRNKEIDELGVITREPF--SELVITDE
 BYSV-CP MAGQND-----EESIDSSAQIMTAKD-----MIFAPPENFARAS-----ATCINGENKKLLKEEFSVRKIQ--DVIES
 BYSV-CPd M--PFQGAELNENRANKSSLEVFSSSEITREKVGKF-----PNNFHKTFYQANPLNDEBLREVLGKGLTEK--TNLKALDE
 CTV-CP MDEIKKLNNKRNKEIEGDDVAEAESSFSSVN-----LHIDP--TLITMAD-----VRQLSTQQAALNRDLPLILGKHPNLPK
 CTV-CPd M--AGYIVLKKTDKKEMDPVSAVPGYKPVIEKGVANRSDALIEGVLSKLDINSIYEDSEKFTGEHLKYVMVIMDTFLL--ENYKIKTE
 Consensus M.....S.....N.....R.....

GLRaV2-CP GLEWFGILLYALAAITTSKVVQRADEDFVFSNERGE--RNVVTEGDLKKVLDGCAFLHFTNKLRIFGRIFFEAYVDFCIAYKHLQL
 GLRaV2-CPd HFVHIIFELIPC AKISTSEKVKY--VGSH--TWVVGKTYVLDWVNRMMSLIKYVGRVNGLRAPFCACEDLYLIVAPIMSEFKI
 BYV-CP NLGIALGLCLYSCATIGTSNKVNVQPTSTFKASFGGKELMTHGINSFLGSKLLLEKPNLRFCRFKFRQYISLRKEVRGLPEI
 BYV-CPd LFWHLAFALIRAAITTSKVVNV--VGAY--EYTI GKKFLWKDWPVEPLIKECMGRNKNPNVRIFCMTFEDAYIVTARSPLKFIN
 BYSV-CP GIPITLGMILYALATLSTSKIDIEIKTFLASAKIDAM--VITTYEDIRNFASLILLRNKNGRVEARITFEESLRFFVRQYKHILINI
 BYSV-CPd DEYHHAFELLRASVSTSEKVEY--RGSY--SYSIDQRKVTVDNPWIEPQKILASKINKPNLRAPFACSEGVLSVARGPDAFGL
 CTV-CP IKDPRIMMLYRLAVKSSSLQSDDAIGITTYTR--EGVE/LSKLWIDWNSKGI GRTINLRWSGRINDALYAPCRQNRNLSG
 CTV-CPd ILLMLIMIQKRLYTISTSKIKTRKIGCI--SVQGLRKLKLVKVPFLISKFIDRETPNARKEACIFEEHLHMAKRRPLDYEN
 ConsensusS.....N.....R.....

GLRaV2-CP NAAELGIPAEBSYLAADFL-GTCKLS ELQQSRMPASMAIKTESGVAWTP--VSNLRQLGR--EVM
 GLRaV2-CPd KAVGKGLPVGKEVYLGADFLSGTSKLSMHDRAVSVAAKAVDFASTGGERKCVSLMDLGR--Y
 BYV-CP ARANKHGLEPACHYLAADFI--STSTELTDLQSR--LLLARENAHTEPSSSP--VTSLAQLGRGLGTGR
 BYV-CPd RTIKRGKIPSGEYFLGADFLNITSVCLNDEKATVLQASRAIDRAWSSSDGKIVSLFDLGR--S
 BYSV-CP ARANKHGI PADSYLAADFV--QTSNLLKHEQAV--LLEGNAVATASSGITRES--AVNLKYL--GSSK
 BYSV-CPd RSVGRGAIPSGEYLGADFLNITCPLMSHDRAVMSASRAALRESAASQIDKKWLSLDPKVAVT
 CTV-CP GSPDLAIGIPAGHYLCADFL--TGCALTDLE--CAVTIQAQEKLLKRGADVV--VINVRQIGKF--NTR
 CTV-CPd KRTITRAGTPIHGLYSLADFLSGSLPGYS EHERGITRASEM LARKGTEAEELINLRDLGK--YL
 ConsensusG.P.....L.ADF.....G.....

b



FIGURES 6A, 6B

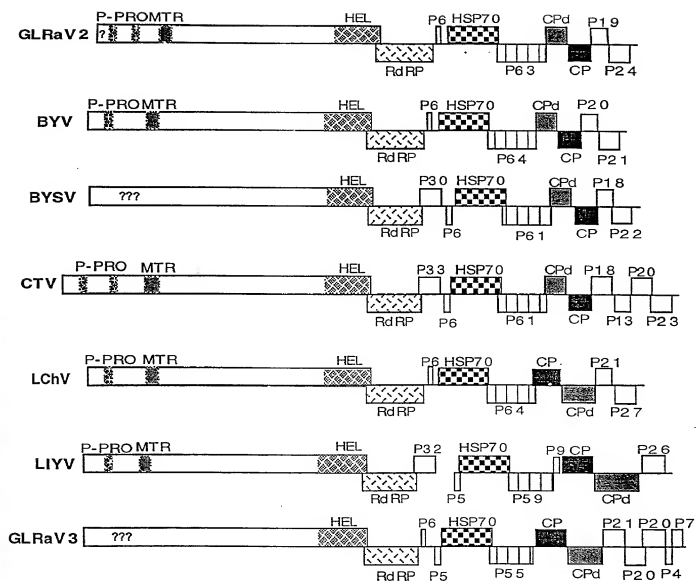


FIGURE 7

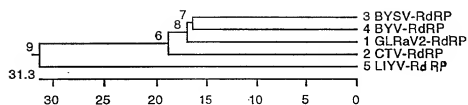


FIGURE 8

09633486.071100

GLRaV2-Hsp90 MS-----NYSWESLFFKIFYGEADWKYLSRSIAHSEIKTLPDIRLYGGRVVKSEFESALP
BYV-Hsp90 MTRFSTPANYWGELFRFFGGQEWKNLMSEAAVSRRPYSS--DFRFSIDGVLIRKFTFGESRG
BYSV-Hsp90 MSRR--PTFAGYSGWGLFKRHYGEPEWKSYLTFETSMKYKPLKSS--STFFDGSGLTSABELRPARS
CTV-Hsp90 MSSH-----HVWGSLFKFYGEAIIWKEYLSESTRNFDERNVSL--DHTLSISGVVVRQSLNAPQ
Consensus M.....W..LF...G...WK.....G.....

GLRaV2-Hsp90 NSFQE--LGLFILSEREVGWS-KLCGITVEEAAYDLTNPKAYKFTAETCSPDVKGEQKYSMED
BYV-Hsp90 ESFVREFSL-LATFPKTYE--VCKLCGVAMELAINGMNLSDYN-VSEFNIVDVKTGCKPFIQIS
BYSV-Hsp90 GT--AEEIETALLIFSDSITKWEKLI-ERSIYRGLAQINNH5IYA--ETELISVDVKTIGCKFTISA
CTV-Hsp90 GTFENE--LAILYNSVVINDFVE--ITGMPLKSLMTGIEDRVF--PDELISVDPEGGRCRFTLND
ConsensusE...L.....L.....L.....L.....D.....H.....G.....

GLRaV2-Hsp90 VMNFMRLSNLNDVNDKMLTEQCWSLSNSCGELINPDDKGRFVALTFKDRDTADDTGAANVECRVGD
BYV-Hsp90 VTEFVKKINGNVAEPLSVLHCWLSNSCGELINPKDKTRFVSLIFPKGKDLAESTDEAIVSSSYLD
BYSV-Hsp90 VESFM--GGRAAAQVEHCWLSNSCGELINPNDTARF IQLVFKDKAVTEQAQ-VNTSGSVSD
CTV-Hsp90 VESYLSRGEADFADLAAVEHSWLSNSCGRLSSTEDDAYKTIVFT--KNF--DSNVSGVITKLET
Consensus V.....E..W.LSNSCG.L.....L.....L.....F.....

GLRaV2-Hsp90 YLVYAMSLFEQRTQKSQSGNISLYEKYCEYIRTYLGSTDLFTTAPDIRPLLTGLYDFCKEYNVF
BYV-Hsp90 YLSHCLNLYETCNLSNSGKKSLYDEFLKHVIDYENSLEYRSPSDNPLVAGILYDMCFEYNTL
BYSV-Hsp90 YLVVYCLQLYDSSKKKSNAGRTQLMESYVSF IRDFFQHSLDYRSPLDNPLTGLVYDLCTIENHVL
CTV-Hsp90 YLSYCI.SLYKHKCMKDD-DYFNLI.LPMFNCLMKVLASLGLFYEKHADNPLTGMLTEECLENKVY
Consensus YL.....L.....L.....L.....L.....L.....PL...G.L...

GLRaV2-Hsp90 YSSYKRNVNFRFFFLANYMPLISDVVFQWVKPAPDV---RLLFELSAEALTLEVPTSLIDSQ
BYV-Hsp90 KSTYLNKIESFDCPLSLYLPLLEVSFNMWERPAPDV---RLLFELDAELLKLVPTINMHDS
BYSV-Hsp90 RGSYLNKLDNFRFLFKQTYLFEMIDIFDYSWELYPDE---RLFPIDPYEIKKEVPTMSVIDAN
CTV-Hsp90 YSTFKVNLNDNVLRFKSKVLEPVLTV---WDISEPDDPMDERVLIPEDPTDFVLDLPKLNHDTM
ConsensusN.....F.....P.....W.....PD.....R.L.....P.....D...

GLRaV2-Hsp90 VVVGHILRYVESYTSDDPAIDALEDKLEAILKSSNPRLSTAQLWVGFGFCYGEFRTAQSRUVQRPFG
BYV-Hsp90 FLYKNKLRYLESYFEDDSNELIKVKVDLSLTRDNPELKLQWRVWGFHCYGVFRTAQTRKVKRDA
BYSV-Hsp90 VVLSNKLNYLDSYLENNSTALEKKIISILCRDNREGIDEGALWAAFFCYGTYRTARQVRVVRPD
CTV-Hsp90 VVVGNIQRLQLEYVVS DALDDLQHVLDLRLAADNPDLRVGLRWAGMFVYGVYRCVVDRAVERPT
ConsensusL.....N.....W.....YYG...R...R.V.R...

GLRaV2-Hsp90 VYKTPDSV-----GGFEINMKDVEKFFDKLQRELPNVSLRRQFNGARAHEAPKIFKNGNISFRP
BYV-Hsp90 EYKLPAL-----GEFVINMSGVEFFELQKMPISVSRRRFCGSLSHFAFVTKRFGVGFPP
BYSV-Hsp90 TYELDGIF-----SKPIV-MSGVELFFDELQKRVDPVLSRRRNFNGAKAGEAIVTFKKLGISFPF
CTV-Hsp90 LFRLPQKLLSQDDGESCSLHMSVEALFNLQVKNKDINVRRQFMGRHSVEVALRLYRNLGLRFPF
ConsensusM..VE..F...Q.....RR.F.G.....A.....F.P

GLRaV2-Hsp90 ISRLNVPRFVWYLNIDYFRHANRSGLTTEEILINNIISVDVRKLCARACN-----TLPSAKR
BYV-Hsp90 ITRLNVVPKYSYLNVDIYRHRKRVGLTQDELITLSNIEFDVAEMCCEREVALQAR--RAQRQEK
BYSV-Hsp90 ITRLNADSKYSYLNIDYFKQANSGLTEPEKIILCNIAKDDVMDMCAQRISSVA-----KP
CTV-Hsp90 ISSVRLPAHHGYLYVDYFVKRVPDGAVTADESLRQLRSVSDVMCKDRVSTPPFPNRLRGSSR
Consensus I.....P.....YL..D.....T..E...L.....V...C...R.....

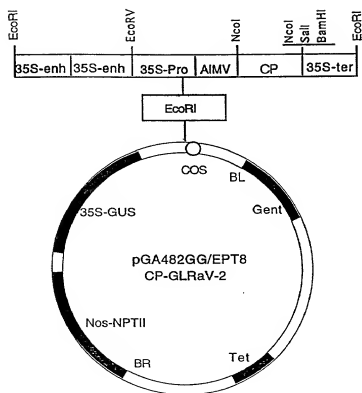
GLRaV2-Hsp90 FSKNHKSNIQSSRQERRIKDPLVLVKDTLYEFQHRKAGWGSSTRDLRGRADHAKGSG.
BYV-Hsp90 IQQWKGTKNEISPHARSSIRVKNNDSLLNLWKDVGARSQRRLNPLHRK-----H
BYSV-Hsp90 IAGRNG--EAINSAKIRTLPTNTLVRALEKCLLNQAPSWMNTTLTNLR
CTV-Hsp90 TFRGARGARGASSRHMSRDVATSGFNLPYHGRLY-----STS
Consensus

GLRaV2 3'-UTR	TTAAGCTGTTACTGAGTAATTAAACCAACAAGTGGTGGTGAATGTGTATGTTGATGTAGA	135
BYS 3'-UTR	TTAAGTCGTACAGAGTGACAACGGCACCAAGTGGTGGTGAATGTGTATGTTGATGTAGA	95
BYSV 3'-UTR	TTAAGCCCTCACAGAGCGAGAACGTTGGCAAGAGCCATTAGTGTGTGTATGTTGATGTAGA	181
CTV 3'-UTR	CTAAGCTCCACAGAGTGGTACTGGTCTCAAGTGAGGCTTAACGTATGCGTGAACCAAGA	208
Consensus	.TAAG.....AC.GAG.....CAAG.G.....T.....A	

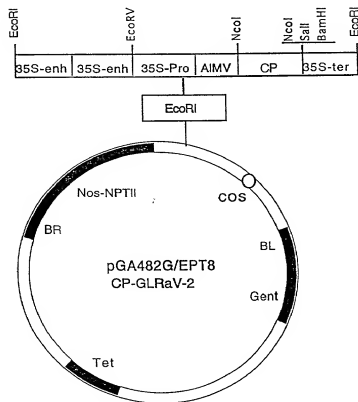
FIGURE 10

09513436-07100

A



B



FIGURES 11A, 11B

09513485-072100



FIGURE 12

00613486, 071100



FIGURE 13

0612486-071100

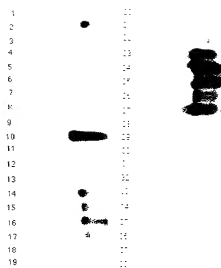


FIGURE 14

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:
GRAPEVINE LEAFROLL VIRUS (TYPE 2) PROTEINS AND THEIR USES

the specification of which (check only one item below):

☒ is attached hereto.

☐ was filed as U.S. Patent Application Serial No. _____ on _____ and was amended on _____ (if applicable).

☐ was filed as PCT International Application Number _____ on _____ and was amended under PCT Article 19 on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specifications, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. 119:

COUNTRY (IF PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
U.S.A.	60/047,194	20/5/97	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO

**COMBINED DECLARATION FOR PATENT
APPLICATION AND POWER OF ATTORNEY (Continued)
(Includes Reference to PCT International Applications)**

**ATTORNEY'S DOCKET NUMBER
19603/1631 (CRF D-2084A)**

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT International filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. 120:

U.S. APPLICATIONS

STATUS (Check One)

U.S. APPLICATION NUMBER

U.S. FILING DATE

PATENTED

PENDING

ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.

**PCT
APPLICATION NO.**

**PCT
FILING DATE**

**U.S. SERIAL NUMBERS
ASSIGNED (if any)**

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. **Michael L. Goldman, Registration No. 30,727, Karla M. Weyand, Registration No. 40,223; Peter Rogalskyj, Registration No. 38,601; Gunnar G. Leinberg, Registration No. 35,584; Dennis M. Connolly, Registration No. 40,964; Edwin V. Merkel, Registration No. 40,087**

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	POST OFFICE ADDRESS	P.O. ADDRESS	CITY	STATE & ZIP CODE/COUNTRY
202	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE/FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	P.O. ADDRESS	CITY	STATE & ZIP CODE/COUNTRY
203	FULL NAME OF INVENTOR	FAMILY NAME	FIRST GIVEN NAME	SECOND GIVEN NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE/FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	P.O. ADDRESS	CITY	STATE & ZIP CODE/COUNTRY

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201

SIGNATURE OF INVENTOR 202

SIGNATURE OF INVENTOR 203

UNSIGNED

UNSIGNED

UNSIGNED

DATE 5/19/98

DATE 5/19/98

DATE 5/19/98